4′-Substituted Nucleosides. 2. Synthesis of the Nucleoside Antibiotic Nucleocidin[1]

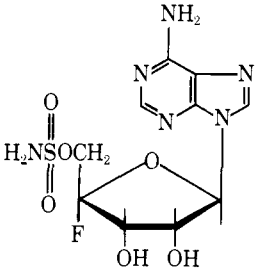
Ian D. Jenkins,[2] Julien P. H. Verheyden, and John G. Moffatt*

Contribution No. 121 from the Institute of Molecular Biology, Syntex Research, Palo Alto, California 94304. Received September 22, 1975

Abstract: The addition of iodine fluoride to N^6,N^6-dibenzoyl-9-(5-deoxy-2,3-*O*-isopropylidene-β-D-*erythro*-pent-4-enofuranosyl)adenine (**9**) leads to the formation of the corresponding 5′-deoxy-4′-fluoro-5′-iodo nucleosides with the β-D-ribofuranosyl (**10**) and α-L-lyxofuranosyl (**11**) configurations. Nucleophilic displacement of the 5′-iodo functions was very difficult but could be accomplished with azide ion giving the 5′-azido-4′-fluoro nucleosides (**18, 18a**). Photolysis of the azides followed by mild hydrolysis and borohydride reduction gave the corresponding 4′-fluoro nucleosides (**20, 20a**) which were converted to their sulfamoyl esters (**22, 22a**) by way of intermediate 5′-*O*-tributyltin (**21, 21a**, R = $SnBu_3$) derivatives. Following removal of protecting groups the nucleoside antibiotic nucleocidin (**1**) and its α-L-lyxofuranosyl epimer **1a** were isolated. Several alternative methods for conversion of the 5′-iodo function in **10** and **11** to the hydroxy counterparts (**20, 20a**) were investigated and this could be accomplished by oxidation with silver(II) oxide followed by borohydride reduction. A number of consistent rules were established for assignment of configuration to 4′-fluoro-2′,3′-*O*-isopropylidene nucleosides from their NMR spectra. A number of other reactions of the 4′-fluoro-5′-iodo nucleosides are also described and some comments concerning the facile cleavage of the pyrimidine ring in N^6-acetyl-N^3,5′-cycloadenosine derivatives are presented.

The microorganism *Streptomyces calvus*, isolated from an Indian soil sample, was shown in 1957 to elaborate an antibiotic substance related to adenosine and referred to as nucleocidin.[3] This substance was shown to exhibit a rather broad antibacterial spectrum and to be particularly active against trypanosomes.[4] Its practical use as either an antibiotic or antitrypanosomal agent is, however, seriously limited by its toxicity, the LD_{50} of nucleocidin in mice being 0.2 mg/kg via intramuscular or intraperitoneal administration.[4] Nucleocidin has been shown to be a highly potent inhibitor of protein biosynthesis, but its precise mechanism of action has not been clearly defined.[5]

Several partial and complete structures were proposed for nucleocidin,[6] but not until 1969 was it realized that the empirical formula properly contained fluorine.[7] With this realization it was possible, by use of NMR and mass spectrometry, to conclude that nucleocidin was correctly represented as 4′-fluoro-5′-*O*-sulfamoyladenosine (**1**) or an isomer thereof.



1

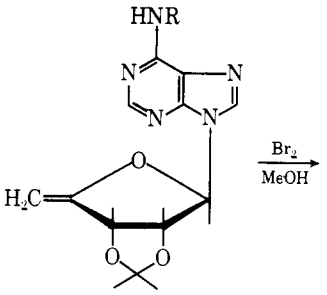
Subsequent work by Shuman et al.[8] supported the β-D configuration for the pentose moiety since upon heating in dimethylformamide nucleocidin was converted to an N^3,5′-anhydronucleoside. Assignment of the D-ribo configuration remained only inferential but is now confirmed by the total synthesis reported in this paper.

The structure **1** for nucleocidin is unique in several ways. Thus, it is, to the best of our knowledge, the first natural product to contain either a fluoro carbohydrate derivative or an unsubstituted sulfamoyl group. It also appears to be the first example of a furanose sugar bearing a functional substituent at C_4. This combination of structural features made the total synthesis of nucleocidin an attractive synthetic challenge, and its successful accomplishment is reported in this paper. A preliminary account of this work has appeared previously.[9]

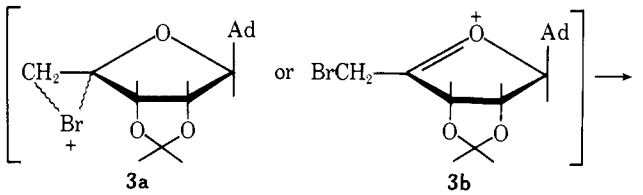
The major synthetic problem in a synthesis of **1** was certainly the stereospecific introduction of the 4′-fluoro function. Previous work in this laboratory has led to the development of general methods for the synthesis of 4′,5′-unsaturated nucleosides in both the pyrimidine[10] and purine series[11] and related studies have been undertaken by others.[12] An attractive route for introduction of the 4′-fluoro moiety appeared to be the addition of a suitable fluorine-containing pseudohalogen such as iodine fluoride to a suitably protected 4′,5′-unsaturated adenosine derivative. Iodine fluoride, which can be isolated from the reaction of iodine and fluorine[13] or can be generated in situ from silver fluoride and iodine, is known to add to olefins[14a] and to vinyl ethers such as glycals.[14b] There was, however, some discouraging precedent for this type of reaction since McCarthy et al.[12b] had attempted the reaction of the olefin **2a** with bromine in methanol. The product of this reaction proved to be the N^3,4′-anhydronucleoside (**4a**), which undoubtedly arose via attack of N^3 of the adenine ring upon $C_{4'}$ of the initially formed 4′,5′-bromonium ion **3a** or its oxonium ion counterpart **3b**.

The above formation of **4a** bears a close resemblance to the well-known, and frequently spontaneous, intramolecular displacement of electronegative $C_{5'}$ substituents leading to N^3,5′-cycloadenosines.[15] Previous work by Jahn[16] has shown, however, that acylation of the 6-amino function in adenosine derivatives reduces the electronegativity of N^3 to the extent that the formation of N^3,5′-cyclonucleosides is greatly suppressed. We have also made considerable use of this observation during various studies on the preparation of 5′-halogenated purine nucleosides,[11] and it seemed worthwhile to first of all examine a model reaction similar to that used by McCarthy et al.[12b] Accordingly, 2′,3′-*O*-isopropylideneadenosine was converted into its N^6-benzoyl derivative **6a**[17] using a modification of the method of Chládek and Smrt.[18] Without purification, crude **6a** (80% yield and 95% purity) was converted essentially quantitatively into its 5′-*O*-methanesulfonyl derivative **6b**. While **6b** could be completely purified by preparative TLC as judged by TLC and NMR analysis, it was not obtained in crystalline form. Treatment of the crude product with an excess of potassium *tert*-butoxide in tetrahydrofuran at −50° readily led to the isolation of crystalline N^6-benzoyl-9-(5-deoxy-2,3-*O*-isopropylidene-β-D-*erythro*-pent-4-enofuranosyl)adenine (**2b**) in an overall yield of 58% from 2′,3′-*O*-isopropylideneadenosine.

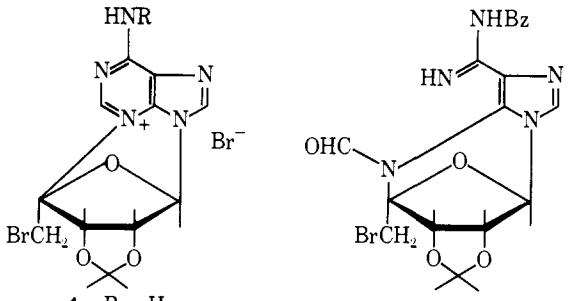
A methanolic solution of the olefin **2b** was then allowed to react at room temperature with a slight excess of bromine



2a, R = H
b, R = Bz

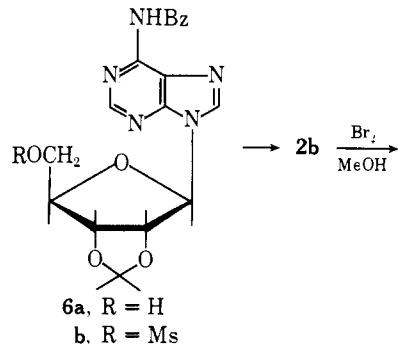


3a 3b

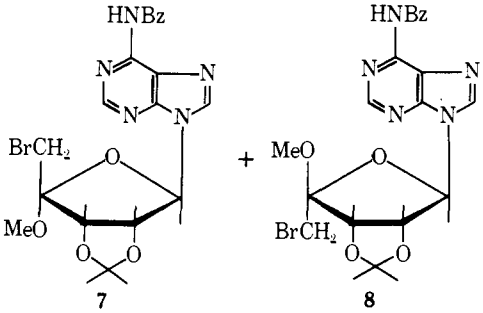


4a, R = H
b, R = Bz 5

giving a mixture of the desired 5′-bromo-4′-methoxy nucleosides **7** and **8** in 46% yield and in a ratio of 2:1 as judged by NMR (see later). By careful preparative TLC **7** and **8** could be separated and isolated as homogeneous foams. Assignments of stereochemistry to **7** and **8** could be made based upon their NMR spectra. Thus the $C_{5'}$ protons in one isomer were magnetically equivalent and appeared as a 2-proton singlet, while in the other isomer these protons were nonequivalent and appeared as a pair of doublets. Since a cis arrangement of the $C_{5'}$-bromomethyl and the 2′,3′-*O*-isopropylidene groups would be expected to inhibit free rotation of the $C_{4'}$–$C_{5'}$ bond, the



6a, R = H
b, R = Ms

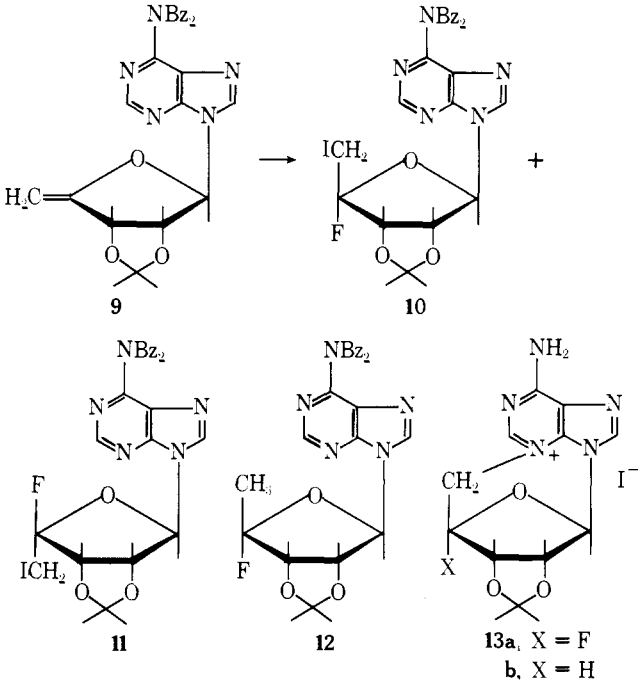


7 8

isomer showing nonequivalent $C_{5'}$ protons is assigned the α-L-lyxofuranose structure **8**. As a further confirmation, a cis relationship between the $C_{5'}$-methylene group and $C_{3'}H$ in the β-D-ribo isomer **7** was demonstrated by a substantial nuclear Overhauser enhancement[19] of the integrated intensity of the $C_{3'}H$ signal upon irradiation of $C_{5'}H$. As will be seen later in this paper, the chemical shift difference between the isopropylidenemethyl signals ($\Delta\delta$ 27 Hz for **7** and 18 Hz for **8**) also confirms the assigned configurations. The presence of the N^6-benzoyl function thus clearly had the desired effect in inhibiting the formation of an N^3,4′-anhydronucleoside. In addition to **7** and **8** the above reaction gave 28% of an unresolved mixture of 5′-bromo-4′-methoxy nucleosides lacking the 2′,3′-*O*-isopropylidene function and only 15% of a compound considered on the basis of elemental analysis and spectral measurements to be the ring-opened N^3,4′-anhydronucleoside **5** rather than the expected **4b**. Recently Sasaki et al.[20] have briefly reported a compound considered to be **5** from the reaction of **2b** with *N*-bromosuccinimide and methanol. The NMR and uv spectra of **5** described by these workers are somewhat different from those we report, but since this compound is peripheral to our central interest we have not repeated the preparation to clarify the situation. Some further comments concerning the spontaneous ring opening of N-acylated cycloadenosine derivatives will be found later in this paper.

The relatively successful reaction of **2b** with bromine and methanol encouraged us to examine the addition of iodine fluoride. In fact, the reaction of **2b** with a mixture of silver fluoride and iodine[14] in tetrahydrofuran did lead to a mixture of fluoroiodo adducts. These compounds, however, had very similar TLC mobilities and could not be efficiently separated. We then considered the possibility that addition of a second benzoyl group to the adenine ring of **2b** might not only further stabilize the molecule against anhydronucleoside formation but also facilitate separation of the isomeric adducts. Accordingly, **2b** was allowed to react in pyridine with benzoyl chloride giving an almost quantitative yield of N^6,N^6-dibenzoyl-9-(5-deoxy-2,3-*O*-isopropylidene-β-D-*erythro*-pent-4-enofuranosyl)adenine (**9**). Such dibenzoyladenine nucleosides have frequently been assigned the N^6,1-dibenzoyl sructure, but recent studies by Anzai and Matsui,[21a] subsequently confirmed by Lyon and Reese,[21b] have shown that the N^6,N^6-dibenzoyl structure is correct. As part of a separate problem to be described later we have also confirmed this conclusion via an examination of the ^{13}C NMR spectra of a number of dibenzoyladenine nucleosides. Complete debenzoylation of **9** with methanolic ammonia gave the known crystalline 9-(5-deoxy-2,3-*O*-isopropylidene-β-D-*erythro*-pent-4-enofuranosyl)adenine (**2a**) in 93% yield.

The reaction of **9** with iodine fluoride generated in situ from silver fluoride and iodine has been examined under a variety of conditions and leads, in most cases, to the formation of a mixture of two isomeric 4′-fluoro-5′-deoxy-5′-iodo nucleosides (**10** and **11**) in 60–90% yield. These two products can be cleanly separated and isolated in analytically pure form by careful preparative TLC using a 40-cm development. On a preparative scale it is more convenient to use chromatography on a column of silicic acid, which effects a partial separation giving pure samples of both **10** and **11** together with some mixed fractions. Rechromatography of the latter permits further resolution and isolation of pure materials. From the NMR spectra of **10** and **11** it was clear that, as expected, fluorine was introduced at $C_{4'}$ of the sugar moiety since both $C_{3'}H$ and $C_{5'}H_2$ showed vicinal coupling to fluorine. This point was confirmed by catalytic hydrogenolysis of the β-D-ribo isomer **10** (see below) which afforded the corresponding 5′-deoxy-4′-fluoro nucleoside **12**, the NMR spectrum of which showed the 5′-methyl group as a 3-proton doublet strongly coupled to the $C_{4'}$-fluorine ($J_{5',F}$ = 17 Hz).



The assignments of configuration for **10** and **11** were based primarily upon NMR spectroscopy and were supported by chemical evidence. The NMR spectra of **10** and **11** are summarized in Tables I and II. The most significant differences in these spectra were the appearance of the $C_{1'}$ proton in the less polar isomer as a doublet of doublets showing $J_{1',2'} = 0.5$ Hz and five-bond long-range coupling to fluorine ($J_{1',F} = 2.5$ Hz). The more polar isomer showed only a small vicinal coupling ($J_{1',2'} \sim 1$ Hz) and no coupling to fluorine. Previous work by Hall et al.[22] on glycofuranosyl fluorides has shown that five-bond H-F coupling ($J_{F',H^4} = 6$–7 Hz) occurs in such compounds when the C_1-F and C_4H functions are trans disposed, whereas the corresponding cis isomers show much smaller values (1–2 Hz). Based upon the above the less polar isomer, which shows $H_{1'}$-F coupling, may be assigned the N^6-dibenzoyl-9-(5-deoxy-4-fluoro-5-iodo-2,3-*O*-isopropylidene-α-L-lyxofuranosyl)adenine structure **11** while the more polar product is the desired β-D-ribofuranosyl isomer **10**. It may be noted that, as expected, $H_{1'}$-F coupling is not observed in the spectrum of nucleocidin itself[7] and the somewhat smaller coupling observed for **11** relative to those in acylated glycofuranosyl fluorides[22] is probably a consequence of the rather rigid conformation induced by the 2′,3′-*O*-isopropylidene function. Indeed, it can be seen from Tables I and II that the spectrum of the α-L-lyxofuranosyl isomer of nucleocidin, derived from **11**, shows a value of $J_{1',F} = 7$ Hz. Similar results have been obtained with some 2′,3′-di-*O*-acyl analogues of **10** and **11** for which values of $J_{1',F} = 0$ and 5–6 Hz, respectively, have been observed.[23]

Further support for these assignments is to be found in an examination of the NMR signals for $C_{3'}$H in **10** and **11**. It has previously been shown[22a,24] in studies on a variety of fluorinated furanose sugars that trans-vicinal H-F couplings are quite large ($J_{H,F} = 20$–30 Hz) while cis-vicinal couplings are much smaller ($J_{H,F} = 4$–10 Hz). In agreement with this the β-D-ribo isomer **10** showed $J_{3',F} = 11.5$ Hz while the cis disposed α-L-lyxo isomer **11** had a considerably smaller $J_{3',F}$ of 5.5 Hz. Once again, the absolute values of these couplings are smaller than those in more flexible acylated sugars due to the isopropylidene group but the relative magnitudes strongly confirm the assigned configurations. Finally, the α-L-lyxo isomer **11** and other related compounds in this paper show a small ($J = 0.5$–1.5 Hz) but clearly apparent "through space" coupling of $C_{4'}$F to C_8H of the purine ring. Such an effect is generally indicative of a physical proximity of the nuclei involved[25] and, since it is not observed in the β-D-ribo isomers (e.g., **10**), adds further support to the above assignments. It should be noted that the $C_{5'}$ protons in most of the 4′-fluoro nucleosides described in this paper give NMR signals that are frequently deceptively simple and not readily amenable to first-order analysis. These ABX patterns have been subjected to computer analysis using established procedures[26] in order to provide the parameters given in Tables I and II.[27]

Chemical support for the above configurational assignments was also sought via formation of an N^3,5′-anhydronucleoside. Thus, small samples of **10** and **11** were first debenzoylated with methanolic ammonia and the resulting nucleosides were directly heated in dimethylformamide. While most 2′,3′-*O*-isopropylideneadenosine derivatives bearing electronegative substituents at $C_{5'}$ form N^3,5′-anhydronucleosides on gentle warming,[15] the presence of the 4′-fluoro function enormously inhibits this reaction. It was necessary to heat the β-D-ribo isomer **10** at 140° for 16 h in order to get roughly 20% conversion to an ionic substance (**13a**) with an ultraviolet spectrum and electrophoretic mobility essentially identical with those of an authentic sample of 2′,3′-*O*-isopropylidene-N^3,5′-cycloadenosine iodide[28] (**13b**). Even under these conditions **11**, in which $C_{5'}$ and the purine ring are trans disposed, failed to give any ionic material similar to **13**.

The relative proportions of **10** and **11** from the condensation of **9** with iodine fluoride varied markedly with the precise reaction conditions. Under many conditions (e.g., slow addition of a solution of 5 equiv of iodine in tetrahydrofuran to a suspension of 6 equiv of silver fluoride and **9** in methylene chloride at room temperature) the predominant product is the undesired α-L-lyxo isomer **11**. In the above experiment the isolated yields of **10** and **11** were 8 and 71%, respectively. Many variations of solvent and condition were explored, ultimately showing that **10** could be obtained as the major product by the gradual addition of solid iodine to a relatively dilute (0.07 M) solution of **9** in methylene chloride at room temperature in the presence of freshly powdered silver fluoride (see the Experimental Section). The optimal conditions in terms of both yield and ratio of products appeared to involve gradual addition of a solution of iodine in acetonitrile to a dilute solution of **9** in acetonitrile in the presence of silver fluoride at −40°. Under a variety of such conditions mixtures of **10** and **11** were obtained in combined yields of 75–85% and in ratios of 1:1 to 2:1. The iodine can be replaced by *N*-iodosuccinimide or the silver fluoride by mercuric fluoride without greatly changing the product distribution. The choice of solvent does not seem to be critical, roughly comparable results arising from reaction in methylene chloride, tetrahydrofuran, acetonitrile, and benzene. The use of nitromethane seems to give a favorable ratio (2:1) of **10** and **11**, but the overall yield is lower (~50%) and a yellow, crystalline by-product is formed that appears to be a mixture of two isomeric nucleosides by NMR. We have not, as yet, been able to characterize this substance.

Since under all conditions investigated a considerable amount of the IF adduct with the undesired α-L-lyxo configuration (**11**) was formed, it was of interest to attempt the equilibration of this product to its more interesting β-D-ribo isomer. It was hoped that in the presence of boron trifluoride **11** would be in equilibrium with the oxonium species **14** which could add fluoride in regenerating a mixture of **10** and **11**. In fact, treatment of an enriched sample of **11** (**11**:**10** = 3:1) with boron trifluoride etherate followed by addition of finely divided sodium fluoride (silver fluoride and tetramethylammonium fluoride seemed to behave similarly) led to the formation of a major product with the same TLC mobility as **10**. Isolation of this material, however, readily gave a crystalline compound identified as N^6-dibenzoyl-9-(5-deoxy-5-iodo-2,3-*O*-isopropylidene-β-D-*erythro*-pent-4-enofuranosyl)adenine (**15**) in

Table I. 100-MHz NMR Chemical Shifts

Compd	Solvent[a]	$C_{1'}H$	$C_{2'}H$	$C_{3'}H$	$C_{4'}H$	$C_{5'}H_a$	$C_{5'}H_b$	C_2H, C_8H	Other
1	P	6.93 (d)	5.25 (dd)	5.56 (dd)		5.03 (d)		8.49, 8.50 (s)	8.23 (br s, 2, NH_2)
1a	P	7.03 (dd)	5.67 (ddd)	~4.90 (m)		4.96 (dd)	5.21 (dd)	8.58, 8.60 (s)	8.20 (br s, 2, NH_2)
2a	P	6.71 (s)	5.50 (d)	5.86 (br d)		4.51 (d)	4.66 (br d)	8.32, 8.56 (s)	1.42, 1.56 (s, 3, CMe_2)
2b	C	6.31 (s)	5.32 (d)	5.55 (dd)		4.53 (d)	4.67 (dd)	8.05, 8.77 (s)	1.42, 1.57 (s, 3, CMe_2), 7.53, 8.02 (m, 5, Ar), 9.15 (s, NH)
5	C	5.38 (s)	4.78 (d)	4.93 (d)		3.99 (d)	4.18 (d)	7.4	1.27, 1.50 (s, 3, CMe_2), 7.4 (m, 4, Ar and C_2H), 8.0 (m, 3, Ar and NCHO)
6a	C	5.96 (d)	5.20 (dd)	5.04 (dd)	4.49 (m)	3.74 (dd)	3.96 (dd)	8.09, 8.70	1.36, 1.62 (s, 3, CMe_2), 7.5, 7.99 (m, 5, Ar)
6b	C	6.16 (d)	5.44 (dd)	5.10 (dd)	4.45 (m)	4.45 (m)	4.45 (m)	8.11, 8.74	1.37, 1.60 (s, 3, CMe_2), 2.89 (s, 3, SO_2Me), 7.5, 7.98 (m, 5, Ar)
7	C	6.21 (d)	5.31 (br s)	5.31 (s)		3.64 (s)		8.11, 8.76 (s)	1.39, 1.66 (s, 3, CMe_2), 3.47 (s, 3, OMe), 7.5 and 8.0 (m, 5, Ar)
8	C	6.51 (br s)	5.55 (br d)	4.92 (d)		3.52 (d)	3.71 (d)	8.13, 8.79 (s)	1.42, 1.60 (s, 3, CMe_2), 2.93 (s, 3, OMe), 7.5 and 8.0 (m, 5, Ar)
9	C	6.27 (br s)	5.26 (d)	5.49 (br d)		4.49 (d)	4.63 (d)	8.05, 8.62 (s)	1.42, 1.56 (s, 3, CMe_2), 7.3, 7.8 (m, 10, Ar)
10	C	6.36 (br s)	5.27 (dd)	5.41 (dd)		3.50 (ABX)[b]	3.54 (ABX)	8.15, 8.67 (s)	1.38, 1.63 (s, 3, CMe_2), 7.4, 7.85 (m, 10O Ar)
11	C	6.48 (dd)	5.58 (dd)	5.08 (dd)		3.54 (ABX)	3.57 (ABX)	8.24 (br s), 8.70 (s)	1.40, 1.58 (s, 3, CMe_2), 7.4, 7.85 (m, 10, Ar)
12	C	6.30 (d)	5.30 (dd)	5.11 (dd)		1.65 (d)		8.13, 8.69 (s)	1.37, 1.62 (s, 3, CMe_2), 7.4, 7.85 (m, 10, Ar)
15	C	6.38 (s)	5.36 (d)	5.59 (d)		5.45 (s)		8.10, 8.60 (s)	1.42, 1.55 (s, 3, CMe_2), 7.4, 7.85 (m, 10, Ar)
16	C	6.37 (br s)	5.32 (br d)	5.54 (dd)		5.32 (d)		8.13, 8.63 (s)	1.40, 1.66 (s, 3, CMe_2), 7.4, 7.8 (m, 10, Ar)
17	C	6.42 (dd)	5.64 (dd)	5.22 (dd)		5.33 (d)		8.23 (br s), 8.66 (s)	1.40, 1.59 (s, 3, CMe_2), 7.35, 7.8 (m, 10, Ar)
18	C	6.38 (br s)	5.34 (dd)	5.55 (dd)		3.61 (d)		7.93, 8.41 (s)	1.41, 1.65 (s, 3, CMe_2), 6.24 (br s, 2, NH_2)
18a	P	6.93 (dd)	6.04 (br d)	5.56 (dd)		3.74 (ABX)	3.79 (ABX)	8.52 (d), 8.80 (s)	1.36, 1.53 (s, 3, CMe_2), 8.44 (br s, 2, NH_2)
20	P	6.90 (br s)	5.53 (dd)	5.90 (dd)		4.19 (d)		8.46, 8.56 (s)	1.43, 1.73 (s, 3, CMe_2), 8.29 (br s, 2, NH_2)
20a	P	6.91 (dd)	5.93 (dd)	5.57 (dd)		4.28 (ABX)	4.38 (ABX)	8.55 (d), 8.76 (s)	1.35, 1.52 (s, 3, CMe_2), 8.34 (br s, 2, NH_2)
21 (R = Ac)	C	6.28 (br s)	5.28 (dd)	5.45 (dd)		4.26 (dd)	4.45 (dd)	7.81, 8.30 (s)	1.39, 1.63 (s, 3, CMe_2), 5.89 (br s, 2, NH_2)
21a (R = Ac)	C	6.51 (dd)	5.61 (dd)	5.13 (dd)		4.50 (ABX)	4.55 (ABX)	8.03 (d), 8.42 (s)	1.41, 1.58 (s, 3, CMe_2), 6.61 (br s, 2, NH_2)
22	P	6.86 (br s)	5.40 (dd)	5.92 (dd)		4.83 (dd)	4.96 (dd)	8.47, 8.52 (s)	1.38, 1.67 (s, 3, CMe_2), 8.39 (br s, 4, NH_2)
22a	P	6.80 (d)	5.95 (d)	5.49 (dd)		4.88 (ABX)	4.92 (ABX)	8.38 (d), 8.64 (s)	1.31, 1.47 (s, 3, CMe_2), 8.31 (br s, 2, NH_2)
23a	P	6.90 (dd)	5.93 (dd)	5.48 (dd)		3.33 (ABX)	3.36 (ABX)	8.54 (d), 8.80 (s)	1.37, 1.54 (s, 3, CMe_2), 8.40 (br s, 2, NH_2)
23b	C	6.47 (br d)	5.52 (d)	5.09 (dd)		3.81 (ABX)	3.86 (ABX)	8.17 (br s), 8.66 (s)	1.40, 1.58 (s, 3, CMe_2), 7.3, 7.8 (m, 10, Ar)
24	D	6.92 (s)	4.85 (d)	5.30 (d)	5.27 (br s)	5.05 (dd)	5.66 (dd)	9.02, 9.40 (s)	1.25, 1.49 (s, 3, CMe_2), 2.50 (s, 3, NAc), 8.02 (s, 1, NH)
25	C	5.83 (s)	4.70 (m)	4.70 (m)	4.70 (m)	3.04 (dd)	4.93 (dd)	7.50 (s)	1.28, 1.50 (s, 3, CMe_2), 2.49 (s, 3, NAc), 8.48 (s, 1, NCHO)

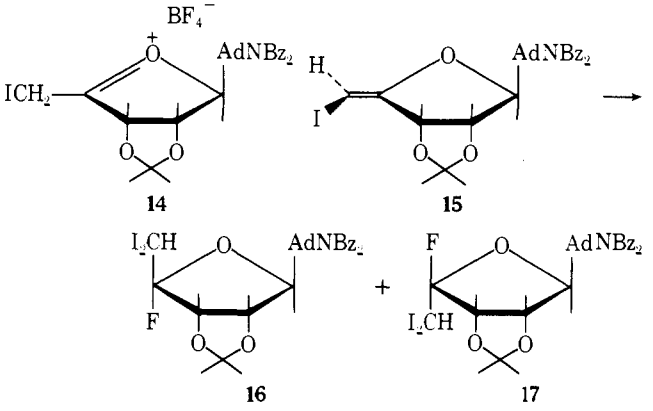
[a] Solvents are: C, $CDCl_3$; P, pyridine-d_5; D, DMF-d_7. [b] The ABX patterns for the $C_{5'}$ protons of **10**, **11**, **18a**, **20a**–**23a**, and **23b** were derived by computer analysis.[26,27]

47% yield. The elemental analysis of **15** clearly indicated the loss of the elements of hydrogen fluoride from **11** and the NMR spectrum showed the absence of a 4′ proton and the presence of only a single vinylic 5′ proton at 5.45 ppm. There was no indication of allylic (3′,5′) coupling, suggesting that these protons are trans oriented relative to the double bond. It is known that cisoid allylic couplings are generally somewhat larger than transoid,[29] and it has been observed that only one of the 5′ protons in nucleosides such as **2a**, **2b**, **9**, or their pyrimidine counterparts[10] is allylically coupled to $C_{3'}H$ ($J_{3',5'b}$ = 1 Hz). The presence of the double bond is also supported by a positive spray test with dilute potassium permanganate on

Table II. First-Order Coupling Constants, Hz[a]

Compd	$J_{1',2'}$	$J_{1',F}$	$J_{2',3'}$	$J_{3',4'}$	$J_{4',5'a}$	$J_{4',5'b}$	$J_{5'a,5'b}$	Other
1	2	0	6	17.5	8.5	8.5	0	
1a	7	7	7	*b*	14	20	10	$J_{2',4'} = 3$
2a	0		6				2	$J_{3',5'a} = 0$, $J_{3',5'b} \sim 1$
2b	0		6				2	$J_{3',5'a} = 0$, $J_{3',5'b} = 1$
5	0		5				13	
6a	4		6	1.5	2.5	2	12.5	
6b	2		6	3	*b*	*b*	*b*	
7	0.5		0				0	
8	<1		5.5				12	
9	<1		6				3	$J_{3',5'a} = 0$, $J_{3',5'b} \sim 1$
10	~1	0	6.5	11.5	18.24	10.26	11.50	
11	0.5	2.5	5.5	5.5	14.54	20.96	13.00	$J_{8,F} \sim 0.5$
12	1	0	6.5	12.5	17	17	0	
15	0		6					
16	~1	0	6	12.5	17.5			
17	1	2.5	5.5	4.5	26.5			$J_{8,F} \sim 0.5$
18	~1	0	6	13	13.5	13.5	0	
18a	0.5	2.5	6	7	19.56	15.44	8	
20	~1	0	6.5	12	9.5	9.5	0	
20a	1	2.5	6	6	10.63	28.37	13.00	$J_{8,F} = 1.5$
21 (R = Ac)	~1	0	6	12	10	12	12	
21a (R = Ac)	1	2	5.5	5.5	22.74	12.76	12.5	$J_{8,F} = 1$
22	<1	0	6	12	12	17	10	
22a	0	2.5	5.5	5.5	13.28	19.22	11	$J_{8,F} = 1.5$
23a	<1	2	6	6	15.39	22.61	12.00	$J_{8,F} = 2$
23b	<1	2.5	5.5	5.5	12.40	21.58	12.50	$J_{8,F} \sim 0.5$
24	0		5	0	3	2	14	
25	0		*b*	*b*	2	2.5	14.5	

[a] The coupling constants for the $C_{5'}$ protons in **10**, **11**, **18a**, **20a**–**23a**, and **23b** were derived by computer analysis.[26,27] [b] Unresolved.



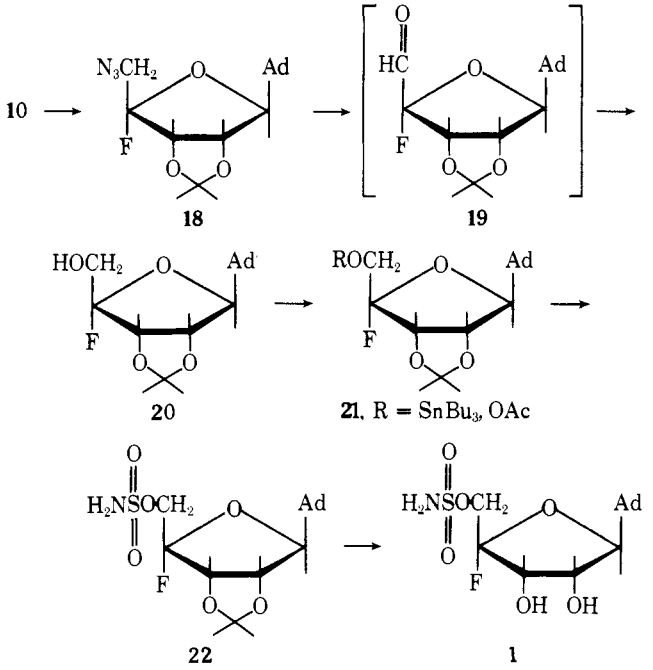
TLC plates. The reaction of **15** with iodine and silver fluoride in methylene chloride gave two readily separated crystalline adducts (**16** and **17**) in yields of 49 and 42%, respectively. The assignments of configuration to **16** and **17** were based upon the same NMR arguments used for **10** and **11**, the ribo isomer **16** showing no 1′,F or 8,F coupling and a large (12.5 Hz) transvicinal $C_{3'}H$-$C_{4'}F$ coupling. On the other hand, **17** showed $J_{1',F} = 2.5$ Hz, $J_{8,F} \sim 0.5$ Hz, and $J_{3',F} = 4.5$ Hz, all in agreement with the indicated assignments. Displacement of the iodine from **16** by reaction with sodium benzoate, lithium azide, or silver trifluoroacetate at 80° for 20 h was not possible.

With the successful introduction of the 4′-fluoro function, the key step in the synthesis of nucleocidin was the seemingly simple conversion of the primary 5′-iodide in **10** to a hydroxyl group. The displacement of the iodine by a wide range of oxygen nucleophiles (e.g., lithium acetate, lithium benzoate, sodium benzoate, silver acetate, dimethyl sulfoxide) under vigorous conditions was, however, uniformly unsuccessful. The only nucleophile that we have successfully used was, in fact, azide ion, the reactions of **10** and **11** with lithium azide in dimethylformamide at 95° for 20 h leading to smooth formation of the corresponding 5′-azido-4′-fluoro nucleosides. Under these vigorous conditions there was concomitant partial loss of one of the N^6-benzoyl functions, and, following completion of this hydrolysis with methanolic ammonia at room temperature, 5′-azido-5′-deoxy-4′-fluoro-2′,3′-*O*-isopropylideneadenosine (**18**) was obtained from **10** in 93% yield (Scheme I). Similar treatment of **11** led to the crystalline α-L-lyxofuranosyl isomer **18a** in 84% yield.

The extraordinary resistance of the iodine function in **10** and **11** is reminiscent of the difficulties that have been observed in the displacement of 1-*O*-tosylketoses and certain 2-*O*-tosyl aldosides with charged nucleophiles.[30] This general effect, occurring in species containing electronegative α-substituents, has been rationalized by Richardson[30a] in terms of both electronic effects and the development of adverse dipolar interactions in the SN2 transition states. The high electronegativity of fluorine clearly has a very strong deactivating effect upon attempted displacement of iodide from **10** or **11**. We have recently shown a similar, but considerably weaker, effect in 5′-deoxy-5′-iodo-4′-methoxy nucleosides.[1]

Further progress toward the synthesis of nucleocidin then depended upon the development of routes for conversion of the 5′-azido function in **18** to a hydroxyl group. One possible route involved catalytic reduction of the azide to an amino group followed by nitrous acid deamination. To test this, the more readily available α-L-lyxofuranosyl azide **18a** was reduced to give the crystalline 5′-amino-4′-fluoro nucleoside **23a**. Unfortunately, however, treatment of either **23a** or its N^6-benzoyl derivative with isoamyl nitrite and acetic acid in dimethylformamide led to complex mixtures, presumably due to side reactions with the 6-nitrogen function. An alternative approach was based upon the work of Doyle and Wierenga[31] which has

Scheme I[a]



[a] Throughout this scheme compounds designated as **18a–22a** and **1a** are identical with **18–22** and **1** above except that the configuration at $C_{4'}$ is inverted (α-L-lyxofuranosyl).

shown that alkyl azides can be converted into the corresponding alcohols via reaction with nitrosonium tetrafluoroborate. This reaction presumably involves the formation and hydration of a carbonium species via loss of nitrous oxide and nitrogen from an initially formed *N*-nitroso azide.[31,32] Attempted application of this reaction to **18a** or to its N^6-benzoyl derivative, however, gave a complex reaction mixture presumably due to side reactions with other nitrogenous functions in the molecule.[33] It may be noted that this reaction is highly effective when applied to the uracil analogue of **18**.[9b]

It is well known that photolysis of an alkyl azide leads to the corresponding imine via loss of nitrogen followed by prototropic rearrangement of the resulting nitrene.[34] Subsequent mild hydrolysis then gives the corresponding carbonyl compound, a reaction that has been applied to carbohydrate derivatives.[35a] More recently this sequence has been applied to several 5′-azido nucleosides although the resulting free 5′-aldehydes have not been isolated as such.[35b] We have found that photolysis of **18** proceeds reasonably well in solvents such as benzene using both high- and low-pressure ultraviolet sources fitted with Pyrex filters. Subsequent mild acidic hydrolysis of the resulting imine then gave a product which upon TLC gave the positive test with 2,4-dinitrophenylhydrazine spray expected for the 5′-aldehyde **19**. The absence of a proton at $C_{4'}$ of **19** would probably obviate most of the problems encountered during chromatography of 2′,3′-*O*-alkylidene nucleoside 5′-aldehydes.[36] Nevertheless, we have not attempted to isolate this compound but have rather directly reduced it with sodium borohydride giving readily crystalline 4′-fluoro-2′,3′-*O*-isopropylideneadenosine (**20**), which was isolated by chromatography on silicic acid. The isolated yield of crystalline **20** was generally only 20–30% from **18** and in general the reaction worked best on a small scale (100 mg). Comparable photolysis of the α-L-lyxo isomer **18a** consistently gave somewhat higher yields (40–50%) of crystalline **20a** and it is not unlikely that the reduced yield in the β-D-ribo series is a reflection of some undefined intramolecular photochemical reaction between the nitrene, or some other $C_{5'}$ intermediate, and the adenine ring. We have not, however, investigated the nature of the by-products in these reactions.

Since the isomeric fluoro alcohols **20** and **20a** are readily separated by preparative TLC, it is possible to directly photolyze mixtures of the fluoro azides **18** and **18a** and to separate the isomeric 4′-fluoro nucleosides **20** and **20a** at that stage. Thus a mixture of the 4′-fluoro-5′-iodo compounds **10** and **11** containing roughly 65% **10** was carried through the azide displacement and photolysis sequence giving the crystalline nucleosides **20** and **20a** in overall yields of 8 and 4% from the olefin **9**. While these overall yields are not particularly high, the pure final products are, nevertheless, readily available. In order to facilitate the isolation of the desired β-D-ribo compound **20**, a somewhat modified procedure can also be used in which the α-L-lyxofuranosyl-4′-fluoro 5′-azide **18a** is isolated by direct crystallization (32% from **9**) from the mixed azides (**18** and **18a**). Subsequent photolysis and reduction of the mother liquors then allows isolation of **20**, once again in an overall yield of 8% from **9**. This method offers the advantage that less material is carried through the photolysis sequence and chromatographic separation of enriched **20** from **20a** is facilitated.

As an alternative to the photolysis procedure we have also observed that the 4′-fluoro-5′-iodo nucleoside **11** can be directly oxidized to the corresponding 5′-aldehyde with freshly prepared silver(II) oxide in aqueous acetic acid buffered with sodium acetate to pH 3.6. We are not aware of any close precedent for this type of oxidation of a primary alkyl iodide to an aldehyde. We have examined the reaction under a number of different conditions (temperature, solvent, etc.) and found it to be rather capricious, the yields fluctuating considerably in apparently similar reactions. Following reduction of the intermediate aldehyde with sodium borohydride and debenzoylation with methanolic ammonium hydroxide, crystalline **20a** was, in fact, isolated in up to an overall yield of 50% from **11**. For unknown reasons the yields once again seemed to be somewhat lower in larger scale reactions, and this particular approach has not been investigated using pure **10**. It has, however, been applied to a mixture of **10** and **11** (about 65% **10**) using freshly prepared silver(II) oxide suspended on Celite.[37] Following borohydride reduction, debenzoylation, and preparative TLC, crystalline **20** and **20a** were isolated in overall yields of 14 and 10% from the mixture of **10** and **11**. Further work is necessary in order to fully control this reaction, but even at this stage it offers a useful alternative to the photolysis route for the preparation of **20** and **20a**.

Previous work by Shuman et al.[38] has shown that 2′,3′-*O*-ethoxymethylideneadenosine can be readily converted into its 5′-*O*-sulfamoyl derivative through reaction with sulfamoyl chloride in dioxane in the presence of sodium hydride. 5′-*O*-Sulfamoyladenosine itself has, in fact, been shown to exhibit biological activities similar to, and only somewhat lower than, those of nucleocidin.[39] While we were able to successfully sulfamoylate 2′,3′-*O*-isopropylideneadenosine using the sodium hydride technique, application of this method to **20** gave only trace amounts of the desired product **22**. Previous work from this laboratory[40] has shown that the conversion of nucleosides into their 2′,3′-*O*-(dibutylstannylene) derivatives provides a convenient method for activation of the 2′(3′) hydroxyl functions for acylation. Similarly, Valade and Pereyere[41] have shown that monoalkoxytin derivatives react with acyl chlorides to form esters. Accordingly, a suspension of **20** in benzene was heated under reflux in the presence of hexabutyldistannoxane giving a clear solution of the 5′-*O*-tributyltin ether (**21**, R = $SnBu_3$). Without isolation this compound was directly allowed to react with an excess of sulfamoyl chloride at 5° giving crystalline 4′-fluoro-2′,3′-*O*-isopropylidene-5′-*O*-sulfamoyladenosine (**22**) in 87% yield. The location of the sulfamoyl moiety at $C_{5'}$ of the nucleoside was assumed by the typical, adenosine-like ultraviolet spectrum of **22** and by the 0.7-ppm downfield shift of the $C_{5'}$ protons relative to those in **20**. A

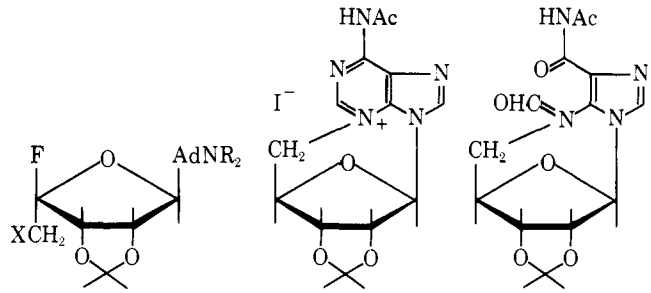
similar sequence applied to **20a** gave the crystalline 5′-*O*-sulfamoyl derivative **22a** in 58% yield. An alternative synthesis of **22a** was also achieved via direct condensation of **20a** with sulfamoyl chloride in dioxane in the presence of Linde 4A or Linde AW-500 molecular sieves to pick up the released hydrogen chloride. A similar approach has been used previously to prepare some 5′-*O*-sulfamoyl derivatives in the uridine series.[38] In view of the excellent yields in the preparation of **22** via the tin ether (**21**, R = $SnBu_3$), we have not explored an alternative method in the ribo series.

Completion of the synthesis of nucleocidin then only required removal of the 2′,3′-*O*-isopropylidene function, a step that was readily accomplished by treatment of **22** with 90% trifluoroacetic acid at room temperature for 30 min. Following this treatment, crystalline nucleocidin (**1**) was obtained as a monohydrate in 77% yield by direct crystallization from water. A crystalline picrate was also prepared with a melting point very similar to that previously described[3] and the NMR spectrum of **1** was essentially identical with that described for the natural product.[7] Most significantly, the antimicrobial spectrum of synthetic **1** was similar to that reported for the natural product,[3] the following minimum inhibitory concentrations (μg/ml) being observed:[39b] *Bacillus subtilis* PCI-219, 12.5; *Bacillus anthracis*, 3.2; *Staphylococcus aureus* FDA-209 P-JC-1, 3.2; *Diplococcus pneumoniae* type I, 0.05; *Streptococcus pyrogenes* C-203, 0.0125; *Escherichia coli* NIHJ JC-2, 1.6; *Klebsiella pneumoniae*, 3.2; *Salmonella typhimurium*, 3.2; *Proteus vulgaris* CN 329, 3.2; *Trichomonas vaginalis* 4F, 1.25. These combined data assure us that the synthetic and natural products are identical and hence provide unequivocal confirmation of the structure of nucleocidin.

It is interesting to note that the hydrolysis of the 2′,3′-*O*-isopropylidene function from the α-L-lyxo compound **22a** was considerably more difficult than in the case of **22**. Under the same conditions used to hydrolyze **22** to **1** in 30 min, complete hydrolysis of **22a** required at least 3 h. Following this treatment crystalline 9-(4-fluoro-5-*O*-sulfamoyl-α-L-lyxofuranosyl)-adenine (**1a**), the 4′-epimer of nucleocidin, was isolated in 87% yield. Since nucleocidin has been considered to function as an analogue of adenosine 5′-phosphate[38] it is, perhaps, not surprising that **1a** showed much reduced biological activity. The difficulty in effecting hydrolysis of the isopropylidene group from **22a** finds close analogy in the resistance toward hydrolysis of 9-(5-deoxy-2,3-*O*-isopropylidene-α-L-lyxofuranosyl)adenine as compared with its β-D-ribofuranosyl isomer.[12b] The precise reason for this stabilizing influence of a cis $C_{5'}$ group is not certain at this time.

Some questions also remain concerning the stabilities of 4′-fluoro nucleosides in general. Thus, we have shown that in the presence of a 2′,3′-*O*-isopropylidene group quite vigorous reactions can be carried out on $C_{5'}$ substituents (e.g., **10** → **18**) without undue problems, and the free 5′-hydroxy derivative **20** is a crystalline and stable product. On the other hand, nucleocidin itself, bearing a 5′-*O*-sulfamoyl substituent, is also a reasonably stable species, and hydrolysis to adenine does not appear to be a problem during acidic removal of the acetonide from **22**. All our efforts to prepare pure, free 4′-fluoroadenosine or its α-L-lyxofuranosyl epimer without substituents on any of the $C_{2'}$-, $C_{3'}$-, or $C_{5'}$-hydroxyl groups have, however, been fruitless. Thus **20** and **20a** have been treated under many different acidic conditions but removal of the isopropylidene group was always accompanied by concomitant release of adenine. The most promising conditions were treatment of **20** with 90% trifluoroacetic acid at room temperature for 6 min and even then roughly 30% adenine was released. Repeated attempts to purify the product by preparative TLC always led to material still contaminated with adenine and one must conclude that 4′-fluoroadenosine undergoes decomposition during chromatography. Continued release of adenine is also apparent upon storage of the partially purified product in aqueous alcohol. Acetylation of **20** readily gave the 5′-*O*-acetyl derivative (**21**, R = Ac) in 90% yield but attempted acidic hydrolysis once again was complicated by simultaneous release of adenine. Related studies on the stabilities of pyrimidine-4′-fluoro nucleosides will be reported elsewhere.[42]

A final possible approach to the direct conversion of the 5′-iodo substituent in **10** or **11** to the 5′-hydroxy analogue (**20**, **20a**) was suggested by the work of Miller and Hoffmann,[43] who have demonstrated that electrolysis of alkyl iodides in acetonitrile leads to the corresponding N-substituted acetamides, presumably via a carbonium ion. We have attempted the electrolysis of **11** using platinum electrodes in acetic acid containing sodium acetate, but under these conditions a conducting solution was not obtained. Upon addition of lithium chloride a conducting solution resulted and iodine was released. A single new product was formed in this reaction and isolated almost quantitatively. While it was not isolated in crystalline form, this substance was clearly shown to be the corresponding 5′-chloro-4′-fluoro nucleoside **23b** by spectroscopic methods. Thus the NMR spectrum of **23b** was essentially identical with that of **11** except that the $C_{5'}$ protons were deshielded by roughly 0.3 ppm. The mass spectrum of **23b** showed the expected molecular ion at *m/e* 551 and 553 and many of the higher mass fragments also showed the typical doubling of peaks due to the presence of stable chlorine isotopes.[44] This reaction was definitely electrolyte in nature since there was no formation of **23b** in the absence of a current. Various attempts to conduct the reaction in the presence of other electrolytes were unsuccessful and this approach has not been explored further.



It should be pointed out that throughout the sequence of reactions described in this paper the NMR characteristics used to assign configurations to **10** and **11** are consistently observed. As will be seen in Table I, all of the 4′-fluoro-2′,3′-*O*-isopropylidene-α-L-lyxofuranosyl compounds exhibited significant (2–2.5 Hz) $C_{1'}H$–$C_{4'}F$ coupling, small (0.5–1 Hz) but readily apparent C_8H–$C_{4'}H$ coupling, and fairly small (5.5–7 Hz) cis-vicinal $C_{3'}H$–$C_{4'}F$ coupling. On the other hand, the β-D-ribo isomers showed no $C_{1'}H$–$C_{4'}F$ or C_8H–$C_{4'}F$ coupling while $J_{3',F}$ was 11.5–13 Hz. It should be noted that in the absence of the 2′,3′-*O*-isopropylidene group the values of $J_{1',F}$ and $J_{2',F}$ are increased to 7 and 3 Hz, respectively, in α-L-lyxofuranosyl nucleocidin (**1a**) while $J_{8,F}$ is barely apparent. Neither coupling is present in nucleocidin itself. The chemical shift difference for the isopropylidene methyl signals is also a consistent indication of configuration at $C_{4'}$, the β-D-ribo compounds showing $\Delta\delta$ = 24–30 Hz while the α-L-lyxo isomers showed $\Delta\delta$ = 16–19 Hz. These chemical shift differences thus become a sensitive indication of configuration at either $C_{1'}$[45] or $C_{4'}$. As yet no examples of compounds in which the aglycon, the isopropylidene group, and $C_{5'}$ are all cis (β-L-lyxofuranosyl) are available.

Finally, the spontaneous hydrolytic cleavage of the pyrimidine ring leading to **5** prompts some comments concerning the stability of N^6-acyl derivatives of the related N^3,5′-anhy-

droadenosine, Mizuno and Sasaki[46] have reported that acylation of the 6-amino group in 2′,3′-*O*-isopropylidene-N^3,5′-cycloadenosine salts (**13b**), giving presumably **24**, activates $C_{5'}$ toward nucleophilic attack and they have used this method for the synthesis of several dinucleoside phosphates. We were interested in the possibility of using this reaction to make N^6-acylated 5′-iodo nucleosides as alternative precursors for **2b**. With this in mind, we reacted the readily available anhydronucleoside **13b**[28] with acetic anhydride and pyridine in dimethylformamide in the presence of an excess of sodium iodide. Following a simple aqueous workup a crystalline product was isolated in 57% yield and shown to be the ring-opened anhydronucleoside **25**. This structure was supported by analytical figures which clearly showed the loss of one nitrogen atom. The *N*-acetyl group was, however, evident in the NMR spectrum as a 3-proton singlet at 2.49 ppm and singlets, similar to those of C_2H and C_8H of the purine ring, were present at 7.50 and 8.48 ppm although the former of these appeared at abnormally high field. This signal is, however, very similar to those shown by C_2H in a variety of 1-substituted 5-amino-4-carboxamidoimidazole derivatives.[45b,47] The latter include some compounds quite closely related to **25** obtained via spontaneous hydrolysis of several N^3,5′-anhydronucleoside derivatives.[47] Hence we ascribe the 1-proton singlet at 8.48 ppm to the *N*-formyl proton in **25**. It should be noted in Table I that the $C_{5'}$ protons in **25** show an unusually large chemical shift difference, being separated by 1.89 ppm. It is not clear why spontaneous hydrolysis of the N^3,4′-anhydronucleoside **4b** led to the amidine derivative **5** while **24** suffered complete loss of N^1 with formation of the amide **25**.

Alternatively, **13b** was treated with pyridine and acetic anhydride in dimethylformamide and, without exposure to water, the product was triturated with ethyl acetate giving the acylated anhydronucleoside **24** in quantitative yield as a yellow powder. This compound was rather unstable and attempted crystallization led to partial decomposition. While its elemental analysis was not within acceptable limits (C and N roughly 1% off) the NMR spectrum of the product showed the presence of a single substance compatible with structure **24**. Attempts to replace the iodide anion by chloride by passage of an aqueous solution of **24** through a column of Bio-Rad AG-1(X2) resin in the chloride form led to spontaneous crystallization of **25** in 50% yield. While we have not explored the conditions necessary for this hydrolysis, it is obvious that N^6-acyl-N^3,5′-anhydronucleosides such as **24** are very susceptible to cleavage of the pyrimidine ring.

The work described in this paper leads not only to the first synthesis of nucleocidin itself but also to the development of a useful general route for the synthesis of 4′-fluoro nucleosides. The synthesis of some other compounds in this series, including some base analogues of nucleocidin, will be described shortly.[42]

Experimental Section

General Methods. Preparative TLC was conducted using 20 × 100 cm glass plates coated with a 1.3-mm layer of Merck silica gel HF and column chromatography using Merck silica with 0.05–0.20-mm particles. Proton nuclear magnetic resonance (NMR) spectra were obtained using a Varian HA-100 spectrometer and are recorded in parts per million downfield of an internal standard of tetramethylsilane. ^{19}F NMR spectra were obtained on a Varian HA-100 instrument operating at 94.1 MHz. Mass spectra were obtained using an Atlas CH-4 spectrometer fitted with a direct inlet system. We are particularly grateful to Dr. M. L. Maddox and Mrs. J. Nelson for their continual help with NMR spectroscopy. Some elemental analyses were obtained from Dr. A. Bernhardt, Elbach über Engelskirchen, Germany, and other instrumental analyses were done by the staff of the Analytical Laboratories of Syntex Research, to whom we extend our thanks. Melting points were obtained using a hot-stage microscope and are corrected.

N^6-Benzoyl-9-(5-deoxy-2,3-*O*-isopropylidene-β-D-*erythro*-pent-4-enofuranosyl)adenine (2b). Benzoyl chloride (45 ml, 0.39 mol) was added dropwise to a stirred solution of 2′,3′-*O*-isopropylideneadenosine (30.7 g, 0.1 mol) in pyridine (300 ml) at 0° and the mixture was then allowed to warm to room temperature over 1 h. After addition of ice, the mixture was evaporated and a solution of the residue in chloroform was washed with aqueous sodium bicarbonate and then water. Following evaporation of the chloroform the residue was dissolved in pyridine (250 ml) and shaken for 20 min with a solution of potassium hydroxide (40 g) in water (250 ml). Acetic acid (35 ml) was then added with cooling, the solvents were evaporated in vacuo, and the residue was partitioned between chloroform and water. The organic phase was further washed with aqueous sodium bicarbonate and water, dried, and evaporated leaving a residue that was triturated with ethyl acetate. The resulting white precipitate was washed with ether and dried in vacuo leaving 33 g (80%) of **6a** that contained roughly 5% of starting material by NMR and TLC using chloroform–methanol (9:1). Without crystallization,[17] a portion of this material (10.3 g, 25 mmol) was dissolved in pyridine (100 ml), cooled to 0°, and treated dropwise with methanesulfonyl chloride (2.42 ml, 31.3 mmol). After stirring for 1 h at 0° ice was added and the solvent was removed in vacuo. A solution of the residue in chloroform was washed with cold aqueous sodium bisulfate, aqueous sodium bicarbonate, and water, dried ($MgSO_4$), and evaporated leaving slightly impure **6b** as a foam in essentially quantitative yield. Attempted preparation of an analytical sample by preparative TLC using chloroform–methanol (9:1) did not afford a crystalline product. This material was thoroughly dried by coevaporation with benzene and then dissolved in freshly purified tetrahydrofuran (250 ml). This solution was cooled with dry ice to about −50° in a drybox under nitrogen and a cold solution of sublimed potassium *tert*-butoxide (8.5 g, 75 mmol) in tetrahydrofuran (50 ml) was slowly added through a septum. The resulting yellow mixture was allowed to warm to room temperature over 1 h with mechanical shaking. The mixture was then taken in roughly 50-ml portions and added separately to a solution of acetic acid (1.5 ml) and sodium acetate (10 g) in a mixture of ice and water (400 ml) in a Waring blender. The resulting white precipitates were combined, collected by filtration, washed with water, and dried in vacuo. Crystallization from benzene–hexane gave 7.32 g (58% overall) of **2b** with mp 151–153°: λ_{max} (MeOH) 230 nm (ε 13 800), 279 (21 400). Anal. Calcd for $C_{20}H_{19}N_5O_4$ (393.39): C, 61.06; H, 4.87; N, 17.80. Found: C, 60.98; H, 4.75; N, 17.80.

Addition of Bromine to 2b. A 10% solution of bromine in methanol was added dropwise to a stirred solution of **2b** (393 mg, 1 mmol) in methanol (10 ml) until a yellow color persisted. The solvent was evaporated and the residue was subjected to preparative TLC using two developments with chloroform–methanol (19:1) giving three bands. Elution of the least polar band gave 233 mg (46%) of a mixture of **7** and **8** in a ratio of 2:1. By careful preparative TLC using two developments with ether–benzene–methanol (50:50:3) pure samples of the individual isomers could be obtained together with some mixed fractions. The more polar isomer (**7**, 75 mg) was a foam: λ_{max} (MeOH) 230 nm (ε 12 700), 279 (19 900); NMR (see Tables I and II); irradiation of $C_{5'}H_2$ led to a 50% increase in the integrated intensity of the $C_{3'}H$ signal. Anal. Calcd for $C_{21}H_{22}BrN_5O_5$ (504.35): C, 50.01; H, 4.40; N, 13.89. Found: C, 49.52; H, 4.48; N, 13.19.

The less polar isomer **8** also had a uv spectrum typical of *N*-benzoyladenosine (λ_{max} 230, 279) and its NMR spectrum is given in Tables I and II. Anal. Calcd for $C_{21}H_{22}BrN_5O_5$ (504.35): C, 50.01; H, 4.40; N, 13.89. Found: C, 49.48; H, 4.08; N, 13.34.

Elution of the middle band gave 75 mg (15%) of **5** as a TLC homogeneous foam: λ_{max} (MeOH) 239 nm (ε 16 400), 312 (16 900); NMR (see Tables I and II); ORD (MeOH) $[\Phi]_{327}$ 0°, $[\Phi]_{313}^{pk}$ 7300°, $[\Phi]_{285}^{tr}$ 3100°, $[\Phi]_{255}^{pk}$ 7200°, $[\Phi]_{225}^{tr}$ 3000°. Anal. Calcd for $C_{20}H_{20}BrN_5O_5$ (490.32): C, 48.99; H, 4.11; N, 14.28. Found: C, 48.78; H, 4.16; N, 13.81.

The most polar band (100 mg, 28%) consisted of a mixture of 5′-bromo-5′-deoxy-4′-methoxy nucleosides lacking the 2′,3′-*O*-isopropylidene group.

N^6,N^6-Dibenzoyl-9-(5-deoxy-2,3-*O*-isopropylidene-β-D-*erythro*-pent-4-enofuranosyl)adenine (9). Benzoyl chloride (2.4 ml, 20 mmol) was added to a solution of **2b** (3.93 g, 10 mmol) in pyridine (20 ml) at 0° and the mixture was then stored at room temperature for 2 h. After addition of some ice the solvent was evaporated and a solution of the residue in chloroform was washed with aqueous sodium bicarbonate and water, dried ($MgSO_4$), and evaporated. The residue was freed from residual pyridine by coevaporation several times with

toluene and then chromatographed on a column of silicic acid (110 g). Elution with chloroform–acetone (19:1) gave 4.7 g (97%) of **9** as a TLC homogeneous foam. An analytical sample was obtained by preparative TLC using two elutions with chloroform–acetone (19:1): λ_{max} (MeOH) 250 nm (ϵ 21 000), 272 (sh, 16 500). Anal. Calcd for $C_{27}H_{23}N_5O_5$ (497.5): C, 65.18; H, 4.66; N, 14.08. Found: C, 65.00; H, 4.76; N, 13.91.

9-(5-Deoxy-2,3-*O*-isopropylidene-β-D-*erythro*-pent-4-enofuranosyl)adenine (2a). A solution of **9** (497 mg, 1 mmol) in half-saturated methanolic ammonia (15 ml) was kept at room temperature for 24 h and then evaporated to dryness. The crystalline residue was purified by preparative TLC using chloroform–acetone (1:1) and crystallization of the material in the major band from chloroform–hexane gave 268 mg (93%) of **2a** with mp 181.5–183° (reported[12b] mp 182–183°): λ_{max} (MeOH) 259 nm (ϵ 14 700); mass spectrum (70 eV) *m/e* 289 (M^+), 274 ($M^+ - CH_3$), 136 (base + 2 H), 135 (base + H). Anal. Calcd for $C_{13}H_{15}N_5O_3$ (289.29): C, 53.95; H, 5.23; N, 24.20. Found: C, 53.94; H, 5.25; N, 24.49.

N^6,N^6-Dibenzoyl-5′-deoxy-4′-fluoro-5′-iodo-2′,3′-*O*-isopropylideneadenosine (10) and Its α-L-Lyxofuranosyl Isomer 11. (a) Iodine (2.54 g, 10 mmol) was added in small portions over 1 h to a suspension of finely powdered silver fluoride (1.52 g, 12 mmol) in dichloromethane (30 ml) containing **9** (1.0 g, 2 mmol). After a further 2 h the mixture was filtered through Celite and the filtrate was washed with saturated aqueous sodium chloride (filtration), sodium thiosulfate, sodium bicarbonate, and water. Following evaporation the residue (1.15 g) was subjected to preparative TLC on twelve 20 × 40 cm plates coated with a 1-mm layer of Merck silica gel HF and run in a lengthwise manner using chloroform–acetone (19:1). This resolved three close-moving ultraviolet absorbing bands that were eluted with acetone. Elution of the fastest band gave 240 mg (19%) of **11** as a foam that could not be obtained in crystalline form: λ_{max} (dioxane) 249 nm (ϵ 23 200), 275 (18 400); see Table I for ^{1}H NMR; ^{19}F NMR −97.3 ppm relative to $CFCl_3$ (dt). Anal. Calcd for $C_{27}H_{23}FIN_5O_5$ (643.5): C, 50.39; H, 3.60; N, 10.88. Found: C, 49.88; H, 3.85; N, 10.41.

Elution of the middle band gave 408 mg (32%) of the pure D-ribo isomer **10** as a homogeneous foam: λ_{max} (dioxane) 249 nm (ϵ 22 100), 275 (17 500); see Table I for ^{1}H NMR; ^{13}F NMR −103.6 ppm relative to $CFCl_3$ (bdt). Anal. Calcd for $C_{27}H_{23}FIN_5O_5$ (643.5): C, 50.39; H, 3.60; N, 10.88. Found: C, 50.26; H, 3.87; N, 10.54.

The minor, most polar band with an R_f very similar to that of **9** contained 77 mg (5%) of an unidentified by-product.

(b) Optimal Conditions. A solution of **9** (5 mmol) in acetonitrile (300 ml) containing freshly powdered silver fluoride (5.0 g, 40 mmol) was vigorously stirred at −40° while a solution of iodine (5.1 g, 20 mmol) in acetonitrile (125 ml) was added dropwise over 1.25 h. The mixture was then allowed to warm to room temperature and evaporated to dryness. The residue was partitioned between chloroform and a mixture of aqueous sodium bicarbonate, sodium chloride, and sodium thiosulfate. The chloroform layer was washed with water, dried ($MgSO_4$), and evaporated leaving 3.5 g of a foam that was roughly chromatographed on a column of silicic acid (chloroform–acetone, 9:1) giving 2.76 g (77% by uv) of a mixture of **10** and **11** in a ratio of 11:6. This mixture was not separated into the pure isomers but was used directly as described later. In various other experiments such mixtures could be separated by chromatography on silicic acid (100 parts by weight) using 2.5% acetone in chloroform. In this way a considerable amount of pure **11** was obtained followed by a mixture of **10** and **11** and then by pure **10**. Rechromatography of the mixed fractions gave further amounts of the pure isomers.

N^6,N^6-Dibenzoyl-5′-deoxy-4′-fluoro-2′,3′-*O*-isopropylideneadenosine (12). A solution of **10** (50 mg, 77 μmol) in methanol (10 ml) was stirred in an atmosphere of hydrogen in the presence of a 10% palladium-on-charcoal catalyst (5 mg) and triethylamine (10 μl, 100 μmol) for 4 h at room temperature. The mixture was then filtered through Celite, the filtrate was evaporated, and the residue was partitioned between ethyl acetate and water. The organic phase was dried ($MgSO_4$) and evaporated leaving a residue that was purified by preparative TLC using chloroform–methanol (96:4). Elution of the major band left **12** as a homogeneous foam: λ_{max} (MeOH) 250 nm (ϵ 23 000), 273 (17 300). See Table I for NMR. Anal. Calcd for $C_{27}H_{24}FN_5O_5$ (517.5): C, 62.66; H, 4.67. Found: C, 62.06; H, 4.80.

Attempted Conversion of 10 and 11 to N^3,5′-Anhydronucleosides. Small samples of pure **10** and **11** (3 mg) were debenzoylated by treatment with saturated methanolic ammonia at room temperature for 18 h giving single spots by TLC using chloroform–methanol (9:1). The solvent was evaporated and solutions of the residues in dimethylformamide (0.1 ml) were sealed in glass capillaries and heated at 140° for 16 h. Examination of the reactions by TLC using chloroform–methanol (4:1), paper chromatography using ethanol–1 M ammonium acetate (5:2), and paper electrophoresis at pH 7.6 showed that the product from **11** remained essentially unchanged while that from **10** led to roughly 20% conversion to a spot with essentially the same mobility as 2′,3′-*O*-isopropylidene-N^3,5′-cycloadenosine hydroiodide (**13b**): λ_{max} 274 nm (cf. λ_{max} 272 for **13b**).[15,28]

N^6,N^6-Dibenzoyl-9-(5-deoxy-5-iodo-2,3-*O*-isopropylidene-β-D-*erythro*-pent-4-enofuranosyl)adenine (15). Boron trifluoride etherate (6.3 ml, 50 mmol) was added to a solution of a crude, roughly 3:1 mixture of **11** and **10** (1.7 g, 2.5 mmol) in dioxane (20 ml) and the mixture was stored at room temperature for 2 h. Finely divided sodium fluoride (3.0 g, 71 mmol) was then added and stirring was continued for 3 h. Following evaporation of the solvent the residue was partitioned between ethyl acetate and aqueous sodium bicarbonate and the organic phase was dried and evaporated. Since partial debenzoylation had occurred the residue was treated at room temperature for 2 h with pyridine (20 ml) and benzoyl chloride (1.16 ml, 10 mmol). After addition of ice the mixture was evaporated and a solution of the residue in chloroform was washed with aqueous sodium bicarbonate and water. Evaporation of the solvent left a residue that was purified by preparative TLC on four plates using chloroform–acetone (96:4). Elution of the major band (R_f similar to **10**) and crystallization from ethanol gave 738 mg (47% from **9**) of **15** with mp 176–177°: λ_{max} (MeOH) 250 nm (ϵ 23 200), 272 (17 900). Anal. Calcd for $C_{27}H_{22}IN_5O_5$ (623.4): C, 52.02; H, 3.56; N, 11.24. Found: C, 51.79; H, 3.68; N, 11.36.

N^6,N^6-Dibenzoyl-5′-deoxy-4′-fluoro-5′-diiodo-2′,3′-*O*-isopropylideneadenosine (16) and Its α-L-Lyxofuranosyl Isomer 17. A solution of **15** (312 mg, 0.5 mmol) in dichloromethane (50 ml) was stirred with silver fluoride (0.5 g, 4 mmol) while solid iodine (0.5 g, 2 mmol) was slowly added over 1 h. The mixture was then stirred for an additional 2 h, filtered through Celite, and washed with aqueous sodium chloride, sodium thiosulfate, sodium bicarbonate, and then water. The organic phase was evaporated and separated into two major components by preparative TLC on four plates using chloroform–acetone (96:4). Elution of the more polar band gave 192 mg (49%) of **16** with mp 130.5–131.5° from ethanol–chloroform: λ_{max} (dioxane) 249 nm (ϵ 25 800), 275 (19 000). See Table I for NMR. Anal. Calcd for $C_{27}H_{22}FI_2N_5O_5$ (769.3): C, 42.15; H, 2.88; N, 9.10. Found: C, 42.33; H, 2.92; N, 9.10.

Elution of the less polar band gave 161 mg (42%) of **17** with mp 132.5–134.5° from ethanol: λ_{max} (dioxane) 249 nm (ϵ 27 100), 274 (20 200). Anal. Calcd for $C_{27}H_{22}FI_2N_5O_5$ (769.3): C, 42.15; H, 2.88; N, 9.10. Found: C, 42.11; H, 2.67; N, 9.00.

5′-Azido-5′-deoxy-4′-fluoro-2′,3′-*O*-isopropylideneadenosine (18). A solution of **10** (480 mg, 0.75 mmol) and lithium azide (150 mg, 3 mmol) in dimethylformamide (7 ml) was heated at 95° for 20 h in the dark. The solvent was evaporated in vacuo and the residue was dissolved in a mixture of methanol (3 ml) and concentrated ammonium hydroxide (3 ml). After storage at room temperature for 18 h the solvents were evaporated and the residue was purified by preparative TLC using chloroform–methanol (88:12). The major ultraviolet absorbing band was eluted giving 250 mg (93%) of chromatographically and spectroscopically pure **18** as a foam that failed to crystallize: λ_{max} (MeOH) 258 nm (ϵ 13 300); ν_{max} (KBr) 2125 cm^{-1} (N_3). See Table I for NMR. Anal. Calcd for $C_{13}H_{15}FN_8O_3$ (350.3): C, 44.57; H, 4.32. Found: C, 44.35; H, 4.42.

9-(5-Azido-5-deoxy-4-fluoro-2,3-*O*-isopropylidene-α-L-lyxofuranosyl)adenine (18a). Treatment of **11** (643 mg, 1 mmol) with lithium azide (200 mg, 4 mmol) and subsequent debenzoylation was carried out exactly as described for **18** above. Following evaporation of the solvents the residue was crystallized from ethyl acetate giving 294 mg (84%) of **18a** with mp 213–214°: λ_{max} (MeOH) 258 nm (ϵ 14 200); ν_{max} (KBr) 2120 cm^{-1} (N_3); ORD (MeOH) $[\Phi]_{280}^{tr}$ −3800°, $[\Phi]_{253}$ 0°, $[\Phi]_{240}^{pk}$ 2000°; see Table I for ^{1}H NMR; ^{19}F NMR (pyridine) C_4-F −105.01 ppm (from $CFCl_3$) dddd, $J_{5'a,F}$ = $J_{5'b,F}$ = 17 (av), $J_{3',F}$ = 6, $J_{1',F}$ = 2.5 Hz. Anal. Calcd for $C_{13}H_{15}FN_8O_3$ (350.3): C, 44.57; H, 4.32. Found: C, 44.44; H, 4.49.

4′-Fluoro-2′,3′-*O*-isopropylideneadenosine (20). A solution of **18** (100 mg, 0.29 mmol) in tetrahydrofuran (5 ml) and benzene (100 ml) was degassed with nitrogen and irradiated in a double walled cylindrical Pyrex reactor fitted with a concentric Westinghouse FS20, 20-W sun lamp while nitrogen was bubbled through the solution. After

18 h the pale yellow solution was evaporated and the residue was treated for 5 min at room temperature with a mixture of dioxane (10 ml) and 0.5 N hydrochloric acid (1 ml). The solution was neutralized with solid sodium bicarbonate and sodium borohydride (150 mg, 0.39 mmol) was added portionwise over 20 min. Excess borohydride was destroyed by careful acidification with acetic acid and the solution was then neutralized with sodium bicarbonate and evaporated to dryness. The residue was coevaporated several times with methanol and then partitioned between chloroform and water. Evaporation of the organic phase left a foam (73 mg) that was separated into two major bands by preparative TLC using chloroform–methanol (9:1). The major, more polar band was eluted and then crystallized from acetone–chloroform giving 24 mg (26%) of **20** with mp 225–226°: λ_{max} (MeOH) 258 nm (ϵ 13 200); ORD (MeOH) $[\Phi]_{280}^{tr}$ −2000°, $[\Phi]_{263}$ 0°, $[\Phi]_{230}^{pk}$ 3800°; mass spectrum (70 eV) *m/e* 325 (M^+), 310 ($M^+ - CH_3$), 267 (M^+ − acetone), 164 (base + 30), 136 (base + 2 H), 135 (base + H); see Table I for NMR. Anal. Calcd for $C_{13}H_{16}FN_5O_4$ (325.3): C, 47.99; H, 4.96; N, 21.53. Found: C, 48.03; H, 4.96; N, 21.82.

9-(4-Fluoro-2,3-*O*-isopropylidene-α-L-lyxofuranosyl)adenine (20a). (a) A sample of **11** (100 mg) was photolyzed using the same procedure described above for **20**. In this case, following the borohydride reduction the nucleosides were desalted by adsorption on charcoal (1.5 g) which was then washed well with water and eluted with 3% ammonium hydroxide in 50% aqueous ethanol. The eluates were then evaporated and purified by preparative TLC using chloroform–methanol (9:1). Elution of the major band and crystallization from ethanol gave 36 mg (39%) of **20a** with mp 236–237°: λ_{max} (MeOH) 258 nm (ϵ 14 400); ORD (MeOH) $[\Phi]_{275}^{tr}$ −2700°, $[\Phi]_{253}$ 0°, $[\Phi]_{240}^{pk}$ 1600°; mass spectrum (70 eV) *m/e* 325 (M^+), 310 ($M^+ - CH_3$), 267 (M^+ − acetone), 247 (*m/e* 267 − HF), 164 (base + 30), 136 (base + 2 H), 135 (base + H); see Table I for NMR. Anal. Calcd for $C_{13}H_{16}FN_5O_4$ (325.3): C, 47.99; H, 4.96; N, 21.53. Found: C, 48.16; H, 5.10; N, 21.58.

In a comparable experiment in which **11** (100 mg) was photolyzed in a mixture of dioxane (1 ml) and benzene (100 ml) using a 450-W Hanovia high-pressure mercury lamp for 6 h a 48% yield of crystalline **20a** was obtained.

(b) A solution of **11** (40 mg, 62 μmol) in dioxane (2 ml) was added to a suspension of silver(II) oxide (0.4 g, 322 μmol)[48] in 1:1 aqueous acetic acid (5 ml) containing sodium acetate (250 mg, final pH 3.6) and the mixture was stirred at room temperature for 16 h. The suspension was then filtered and the filtrate was evaporated leaving a residue that was dissolved in chloroform and washed with aqueous sodium bicarbonate. The dried ($MgSO_4$) organic phase was evaporated and a solution of the residue in aqueous dioxane (1:1, 2 ml) was treated with sodium borohydride (23 mg, 600 μmol) at room temperature for 15 min. The reaction was then acidified with acetic acid, neutralized with sodium bicarbonate, and evaporated to dryness. The residue was dissolved in methanol (1 ml) and concentrated ammonium hydroxide (1 ml) and heated in a sealed tube at 100° for 1 h. The solvents were then evaporated and the residue was roughly purified by passage through a short column of silicic acid in methanol and then subjected to preparative TLC using chloroform–methanol (9:1). Elution of the major band gave 10.5 mg (50%) of **20a** identical with that from (a) above.

Preparations of 20 and 20a without Isolation of Intermediates. (a) Preparation and Photolysis of Mixed Azides. A mixture of **10** and **11** (~65:35) derived from addition of iodine fluoride to 2.5 mmol of **9** was treated with 0.6 g, 12 mmol of lithium azide in dimethylformamide (20 ml) at 100° for 20 h. The solvent was evaporated, the residue was partitioned between chloroform and water, and the organic-soluble material was treated with methanol–concentrated ammonium hydroxide (1:1) at room temperature for 18 h. Benzamide and related by-products were removed by filtration through silicic acid (50 g) using chloroform and then ethyl acetate giving a crude oily mixture of **18** and **18a**. A solution of the mixture in dioxane (5 ml) was added to benzene (1300 ml) and photolyzed with stirring and degassing with argon for 5.5 h using a Rayonet low-pressure reactor. Following evaporation of the solvents a solution of the residue in dioxane (20 ml) was treated for 5 min at room temperature with 1 N hydrochloric acid (7 ml). The solution was neutralized with 1 N potassium hydroxide and then treated with sodium borohydride (400 mg) for 15 min. The mixture was evaporated, filtered through a small column of silicic acid with acetone–methanol (9:1), and purified by preparative TLC using chloroform–methanol (9:1). Rechromatography of the product bands then gave 67 mg of pure **20** and 33 mg of pure **20a**, the overall yields from **2b** being 8 and 4%, respectively.

(b) With Separation of Crystalline 18a. A mixture of crude **10** and **11** (roughly 1:1) obtained from 2.3 mmol of **9** was treated as above with lithium azide (0.4 g) in dimethylformamide (20 ml) at 100° for 20 h. After debenzoylation with methanolic ammonium hydroxide the crude product was crystallized from ethyl acetate giving 256 mg (32% from **9**) of the pure α-L-lyxo azide (**18a**). The mother liquors were evaporated, dissolved in aqueous ethanol (1:1, 200 ml), photolyzed for 45 h, and then hydrolyzed and reduced with sodium borohydride as in (b) above. Preparative TLC of the mixture using chloroform–methanol (9:1) gave a well-defined band corresponding to **20** together with a number of other unidentified materials. Elution of the product band and crystallization from methanol gave 62 mg (8% from **9**) of pure **20** with mp 225–226°.

(c) Reaction of a Mixture of 10 and 11 with Silver(II) Oxide. A solution of sodium hydroxide (20 g) in water (20 ml) was added to a stirred suspension of Celite (8 g) in a solution of silver nitrate (8.5 g) and potassium persulfate (3.5 g) in water (90 ml). The mixture was heated to 100° for 15 min and then cooled. The black suspension was collected by filtration, washed with dilute sodium hydroxide and then with water, and dried in vacuo giving 12 g of silver(II) oxide on Celite (1:2) as a gray powder that was used directly.

A crude mixture of **10** and **11** (644 mg, 1 mmol) containing roughly 65% **10** was dissolved in a mixture of acetic acid (50 ml), 10% aqueous sodium acetate (50 ml), and dioxane (10 ml). The preformed preparation of silver(II) oxide on Celite (1:2, 10.0 g) was added and the mixture was stirred vigorously for 5 h at room temperature and then filtered. The filtrate was evaporated and the residue was partitioned between chloroform and saturated aqueous sodium chloride. The organic phase was washed with aqueous sodium bicarbonate, reduced with sodium borohydride (440 mg) as above, hydrolyzed for 16 h at room temperature with methanol–concentrated ammonium hydroxide, and filtered through a small column of silicic acid (20 g) in acetone. Preparative TLC using chloroform–methanol (9:1) separated **20** and **20a** from two unidentified less polar products and a considerable amount of adenine. Elution of the somewhat less polar band and crystallization from ethanol gave 45 mg (14%) of the ribo isomer **20** while similar treatment of the more polar α-L-lyxo isomer gave 32 mg (10%) of crystalline **20a**, both identical with authentic samples from above.

4′-Fluoro-2′,3′-*O*-isopropylidene-5′-*O*-sulfamoyladenosine (22). A suspension of **20** (65 mg, 0.2 mmol) in benzene (6 ml) containing hexabutyldistannoxane (240 mg, 0.65 mmol) was heated under reflux under anhydrous conditions for 2 h. The resulting clear solution was cooled to 5° in a drybox under nitrogen and a solution of sulfamoyl chloride (92 mg, 0.8 mmol) in dioxane (3 ml) was added dropwise. After stirring for an additional 10 min the solvent was evaporated and the residue was extracted with hot hexane. The insoluble residue was quickly treated with dilute methanolic ammonia, evaporated, and purified by preparative TLC using chloroform–methanol (88:12). Elution of the major ultraviolet absorbing band followed by crystallization from acetone–chloroform gave 70 mg (87%) of homogeneous **22** with mp 143–145°. An analytical sample from water gave the hemihydrate with mp 162–165°: λ_{max} (MeOH) 259 nm (ϵ 13 600); see Table I for NMR. Anal. Calcd for $C_{13}H_{17}FN_6O_6S \cdot 0.5H_2O$ (413.4): C, 37.79; H, 4.39; N, 20.34. Found: C, 37.51; H, 4.09; N, 20.20.

9-(4-Fluoro-2,3-*O*-isopropylidene-5-*O*-sulfamoyl-α-L-lyxofuranosyl)adenine (22a). (a) The fluoro alcohol (**20a**, 130 mg, 0.40 mmol) was treated with hexabutylstannoxane and sulfamoyl chloride as described for the preparation of the β-D-ribo isomer **22**. Preparative TLC and crystallization of the major band from water gave 90 mg (53%) of **22a** as the hemihydrate with mp 141–143°: λ_{max} (MeOH) 259 nm (ϵ 15 300). Anal. Calcd for $C_{13}H_{17}FN_6O_6 \cdot 0.5H_2O$ (413.4): C, 37.79; H, 4.39; N, 20.34. Found: C, 37.84; H, 4.78; N, 20.32.

(b) A solution of **20a** (40 mg, 123 μmol) and sulfamoyl chloride (45 mg, 375 μmol) in anhydrous dioxane (2 ml) was stirred for 18 h at room temperature in the presence of Linde 4A molecular sieve (0.5 g).[49] The mixture was then neutralized by dropwise addition of dilute ammonium hydroxide and evaporated to dryness. The residue was dissolved in methanol, filtered, and purified by preparative TLC using chloroform–methanol (9:1). Elution of the major band gave 29 mg (58%) of **22a** as a foam that was chromatographically homogeneous and identical with that from (a) above.

4′-Fluoro-5′-*O*-sulfamoyladenosine (1, Nucleocidin). A solution of

22 (162 mg, 0.4 mmol) in 90% aqueous trifluoroacetic acid (7.5 ml) was kept at room temperature for 30 min and then evaporated to dryness. The residue was dissolved in methanolic ammonium hydroxide (1:1, 5 ml) and evaporated to dryness leaving a residue that was crystallized from water giving 113 mg (77%) of **1** as a monohydrate which slowly decomposed above 190° (the melting point of free nucleocidin does not appear to have been described previously). A crystalline picrate was prepared with mp 145–147° (reported[3] mp 143–144°): λ_{max} (MeOH) 259 nm (ϵ 15 100); ORD (MeOH) $[\Phi]_{280}^{tr}$ −3900°, $[\Phi]_{257}$ 0°, $[\Phi]_{235}^{pk}$ 1400°; see Table I for NMR. Anal. Calcd for $C_{10}H_{13}FN_6O_6S \cdot H_2O$ (382.34): C, 31.41; H, 3.95; N, 21.98. Found: C, 31.52; H, 3.41; N, 21.73.

9-(4-Fluoro-5-*O*-sulfamoyl-α-L-lyxofuranosyl)adenine (1a). A solution of **22a** (97 mg, 0.23 mmol) in 90% aqueous trifluoroacetic acid (3 ml) was kept at 20° for at least 3 h and then worked up as described for **1**. Crystallization from water gave 74 mg (87%) of **1a** which gradually melted with decomposition at 200–201°: λ_{max} (0.01 N HCl) 257 nm (ϵ 14 900); λ_{max} (0.001 N NaOH) 259 nm (ϵ 14 200);[50] ORD (MeOH) $[\Phi]_{280}^{tr}$ −5800°, $[\Phi]_{256}$ 0°, $[\Phi]_{245}^{pk}$ 1700°, $[\Phi]_{229}$ 0°. Anal. Calcd for $C_{10}H_{13}FN_6O_6S$ (364.32): C, 32.97; H, 3.60; N, 23.07. Found: C, 33.25; H, 3.93; N, 22.58.

5′-*O*-Acetyl-4′-fluoro-2′,3′-*O*-isopropylideneadenosine (21, R = Ac). A solution of **20** (32.5 mg, 0.1 mmol) in pyridine (1 ml) and acetic anhydride (0.05 ml) was kept at room temperature for 18 h before being quenched with methanol and evaporated to dryness. The residue was coevaporated twice with toluene and then crystallized from methanol giving 20 mg of pure **21**, R = Ac, with mp 211–212°. The mother liquors were purified by preparative TLC giving a further 9 mg (total yield 29 mg, 79%) of pure product: λ_{max} (MeOH) 259 nm (ϵ 14 500). Anal. Calcd for $C_{15}H_{18}FN_5O_5$ (367.3): C, 49.04; H, 4.94; N, 19.07. Found: C, 49.18; H, 5.17; N, 19.21.

Similar acetylation of a 10-mg sample of **20a** gave essentially complete conversion to the 5′-*O*-acetate (**21a**, R = Ac) that was isolated by preparative TLC but only characterized by NMR (see Table I).

Attempted Electrolysis of 11. A solution of **11** (40 mg, 62 μmol) in glacial acetic acid (30 ml) containing 0.5 M sodium acetate and sufficient lithium chloride to give a conducting solution was electrolyzed between two platinum electrodes (2 cm^2) using a potential of 2.0 V and with a current of 0.03 mA for 2 h. The mixture was then evaporated and a solution of the residue in chloroform was washed with aqueous sodium bicarbonate, sodium thiosulfate, and water. The organic layer was dried and evaporated and the residue was purified by preparative TLC using chloroform–acetone (96:4). Essentially a single ultraviolet absorbing band with a mobility slightly smaller than that of **11** was observed and elution gave 34 mg (quantitative) of **23b** as a foam. The NMR spectrum (see Table I) was essentially identical with that of **11** except for the chemical shift of the $C_{5'}$ protons; mass spectrum (70 eV) *m/e* 551, 553 (M^+), 536, 538 ($M^+ - CH_3$), 523, 525 ($M^+ - CO$), 495, 497 ($M - 2\,CO$), 446, 448 ($M^+ - C_6H_5CO$), 418, 420 (*m/e* 446, 448 − CO).

9-(5-Amino-5-deoxy-4-fluoro-2,3-*O*-isopropylidene-α-L-lyxofuranosyl)adenine (23a). A solution of **18a** (200 mg, 0.57 mmol) in ethanol (20 ml) was vigorously stirred in an atmosphere of hydrogen for 12 h in the presence of a 10% palladium-on-carbon catalyst (20 mg). The mixture was filtered through Celite, evaporated, and purified by preparative TLC using chloroform–methanol (88:12). Elution of the major band and crystallization from ethanol gave pure **23a** with mp 216–219°: λ_{max} (MeOH) 257 nm (ϵ 14 800). See Table I for NMR. Anal. Calcd for $C_{13}H_{17}FN_6O_3$: C, 48.14; H, 5.28; N, 25.92. Found: C, 47.87; H, 5.33; N, 25.73.

4-(*N*-Acetylcarbamoyl)-5′,*N*^{5}-anhydro-5-formamido-1-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)imidazole (25). (a) A solution of **13b** (417 mg, 1 mmol)[28] and sodium iodide (1.5 g, 10 mmol) in a mixture of dimethylformamide (10 ml), pyridine (0.1 ml, 1.25 mmol), and acetic anhydride (1 ml, 10 mmol) was kept at room temperature for 3 days. Following evaporation of the solvents the residue was suspended in chloroform and washed four times with water and then with aqueous sodium thiosulfate. The solvent was evaporated and the residue was crystallized from methanol (3 ml) giving 200 mg (57%) of **25** with mp 200–201°: λ_{max} (MeOH) 217 nm (ϵ 25 600), 266 (11 500); ORD (MeOH) $[\Phi]_{303}$ 0°, $[\Phi]_{291}^{pk}$ 380°, $[\Phi]_{284}$ 0°, $[\Phi]_{272}^{tr}$ −1500°, $[\Phi]_{261}$ 0°, $[\Phi]_{248}^{pk}$ 1600°, $[\Phi]_{218}$ 0°; see Tables I and II for NMR. Anal. Calcd for $C_{15}H_{18}N_4O_5$ (350.32): C, 51.42; H, 5.18; N, 15.99. Found: C, 51.12; H, 5.04; N, 16.17.

(b) A solution of **13b** (2.085 g, 5 mmol) in dimethylformamide (25 ml), acetic anhydride (5 ml), and pyridine (0.5 ml) was stored overnight at room temperature and then evaporated to dryness. After thorough drying at 50° under high vacuum the residue was triturated with ethyl acetate giving 2.4 g (quantitative) of **24** as a dry solid (see Tables I and II for NMR): λ_{max} (MeOH) 220 nm (ϵ 24 800), 272 (16 000); ORD (MeOH) $[\Phi]_{280}^{tr}$ −3100°, $[\Phi]_{259}$ 0°, $[\Phi]_{222}^{pk}$ 7800°. This material (400 mg) was dissolved in water (3 ml) and passed through a column containing 8 ml of Bio-Rad AG-1 (X2) resin in the chloride form, the resin being further washed with water (25 ml). Partial evaporation of the water led to crystallization of 165 mg (50%) of **25** identical with that described above.

Acknowledgments. The authors are grateful to Mr. K. Knopes and Mr. T. Tompkins for repeating some part of this work on a larger scale.

References and Notes

(1) For part I see J. P. H. Verheyden and J. G. Moffatt, *J. Am. Chem. Soc.*, **97**, 4386 (1975).
(2) Syntex Postdoctoral Fellow, 1969–1971.
(3) S. O. Thomas, V. L. Singleton, J. A. Lowery, R. W. Sharpe, L. M. Pruess, J. N. Porter, J. H. Mowat, and N. Bohonos, *Antibiot. Annu.*, 716 (1956–1957).
(4) R. I. Hewitt, A. R. Gumble, L. H. Taylor, and W. S. Wallace, *Antibiot. Annu.*, 722 (1956–1957).
(5) (a) J. R. Florini, H. H. Bird, and P. H. Bell, *J. Biol. Chem.*, **241**, 1091 (1966); (b) J. R. Florini in "Antibiotics", Vol. I, I. D. Gottleib and P. D. Shaw, Ed., Springer-Verlag, New York, N.Y., 1967, p 427.
(6) (a) C. W. Waller, J. B. Patrick, W. Fulmor, and W. E. Meyer, *J. Am. Chem. Soc.*, **79**, 1011 (1957); (b) J. B. Patrick and W. E. Meyer, Abstracts, 156th National Meeting of the American Chemical Society, Atlantic City, N.J., Sept 1968, MEDI 024.
(7) G. O. Morton, J. E. Lancaster, G. E. Van Lear, W. Fulmor, and W. E. Meyer, *J. Am. Chem. Soc.*, **91**, 1535 (1969).
(8) D. A. Shuman, R. K. Robins, and M. J. Robins, *J. Am. Chem. Soc.*, **91**, 3391 (1969).
(9) (a) I. D. Jenkins, J. P. H. Verheyden, and J. G. Moffatt, *J. Am. Chem. Soc.*, **93**, 4323 (1971); (b) J. P. H. Verheyden, I. D. Jenkins, G. R. Owen, S. D. Dimitrijevich, C. M. Richards, P. C. Srivastava, N. Le-Hong, and J. G. Moffatt, *Ann. N.Y. Acad. Sci.*, **255**, 151 (1975).
(10) (a) J. P. H. Verheyden and J. G. Moffatt, *J. Am. Chem. Soc.*, **88**, 5684 (1966); (b) *J. Org. Chem.*, **39**, 3573 (1974).
(11) S. D. Dimitrijevich, J. Smejkal, J. P. H. Verheyden, and J. G. Moffatt, manuscript in preparation.
(12) (a) M. J. Robins, J. R. McCarthy, and R. K. Robins, *J. Heterocycl. Chem.*, **4**, 313 (1967); (b) J. R. McCarthy, R. K. Robins, and M. J. Robins, *J. Am. Chem. Soc.*, **90**, 4993 (1968).
(13) M. Schmeisser, P. Sartori, and D. Naumann, *Chem. Ber.*, **103**, 880 (1970).
(14) (a) L. D. Hall and D. L. Jones, *Can. J. Chem.*, **51**, 2902 (1973); (b) L. D. Hall and J. F. Manville, *Chem. Commun.*, 35 (1968).
(15) V. M. Clark, A. R. Todd, and J. Zussman, *J. Chem. Soc.*, 2952 (1951).
(16) W. Jahn, *Chem. Ber.*, **98**, 1705 (1965).
(17) (a) R. S. Ranganathan, G. H. Jones, and J. G. Moffatt, *J. Org. Chem.*, **39**, 290 (1974); (b) S. David and J.-C. Fischer, *Bull. Soc. Chim. Fr.*, 3610 (1972).
(18) S. Chládek and J. Smrt, *Collect. Czech. Chem. Commun.*, **29**, 214 (1964).
(19) J. H. Noggle and R. E. Schirmer, "The Nuclear Overhauser Effect: Chemical Applications", Academic Press, New York, N.Y., 1971.
(20) T. Sasaki, K. Minamoto, and K. Hattori, *J. Am. Chem. Soc.*, **95**, 1350 (1973).
(21) (a) K. Anzai and M. Matsui, *Bull. Chem. Soc. Jpn.*, **46**, 3228 (1973); (b) P. A. Lyon and C. B. Reese, *J. Chem. Soc., Perkin Trans. 1*, 2645 (1974).
(22) (a) L. H. Hall, P. R. Steiner, and C. Pedersen, *Can. J. Chem.*, **48**, 1155 (1970); (b) K. Bock, C. Pedersen, and L. Wiebe, *Acta Chem. Scand.*, **27**, 3586 (1973).
(23) S. D. Dimitrijevich, I. D. Jenkins, J. P. H. Verheyden, and J. G. Moffatt, manuscript in preparation.
(24) (a) A. B. Foster, R. Hems, and L. D. Hall, *Can. J. Chem.*, **48**, 3937 (1970); (b) J. S. Brimacombe, A. B. Foster, R. Hems, J. H. Westwood, and L. D. Hall, *ibid.*, **48**, 3946 (1970); (c) L. D. Hall and P. R. Steiner, *ibid.*, **48**, 2439 (1970).
(25) See, e.g., (a) K. L. Servis and K. N. Fang, *J. Am. Chem. Soc.*, **90**, 6712 (1968); (b) G. W. Gribble and J. R. Douglas, *ibid.*, **92**, 5764 (1970), and references cited therein.
(26) P. L. Corio in "Structure of High-Resolution NMR Spectra", Academic Press, New York, N.Y., 1967, p 299.
(27) These analyses were kindly done by Dr. M. L. Maddox, to whom we extend our warmest thanks.
(28) J. P. H. Verheyden and J. G. Moffatt, *J. Org. Chem.*, **35**, 2319 (1970).
(29) L. M. Jackman and S. Sternhell in "Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry", 2nd ed, Pergamon Press, New York, N.Y., 1969, p 323.
(30) See (a) A. C. Richardson, *Carbohydr. Res.*, **10**, 395 (1969); (b) D. H. Ball and F. W. Parrish, *Adv. Carbohydr. Res.*, **24**, 139 (1969), and references cited therein.
(31) M. P. Doyle and W. Wierenga, *J. Am. Chem. Soc.*, **94**, 3896, 3901 (1972).
(32) R. H. McGirk and E. H. White, *J. Am. Chem. Soc.*, **95**, 3804 (1973).
(33) M. P. Doyle, W. Wierenga, and M. A. Zaleta, *J. Org. Chem.*, **37**, 1597 (1972).
(34) (a) F. D. Lewis and W. H. Saunders in "Nitrenes", W. Lwowski, Ed., Interscience, New York, N.Y., 1970, p 47; (b) R. A. Abramovich and B. A. Davis, *Chem. Rev.*, **64**, 149 (1964).
(35) See, e.g., (a) D. M. Clode and D. Horton, *Carbohydr. Res.*, **12**, 477 (1970); (b) D. C. Baker and D. Horton, *ibid.*, **21**, 393 (1972).
(36) G. H. Jones and J. G. Moffatt, Abstracts of the 158th National Meeting of

the American Chemical Society, New York, N.Y., Sept 1969, CARB 16.
(37) The oxidation of alcohols and certain nitrogen functions using silver carbonate on Celite is well documented. See, e.g., M. Fetizon, M. Golfier, and J. M. Louis, *Tetrahedron*, **31**, 171 (1975), and references cited therein.
(38) D. A. Shuman, M. J. Robins, and R. K. Robins, *J. Am. Chem. Soc.*, **92**, 3434 (1970).
(39) (a) A. Bloch and C. Coutsogeorgopoulos, *Biochemistry*, **10**, 4394 (1971). (b) Unpublished studies from Shionogi Research Laboratories, Osaka, Japan. We are most grateful to Drs. K. Katagiri, K. Sato, and K. Yamaguchi for these studies.
(40) D. Wagner, J. P. H. Verheyden, and J. G. Moffatt, *J. Org. Chem.*, **39**, 24 (1974).
(41) J. Valade and M. Pereyere, *C. R. Hebd. Seances Acad. Sci.*, **254**, 3693 (1962).
(42) G. R. Owen, J. P. H. Verheyden, and J. G. Moffatt, manuscript in preparation.
(43) L. L. Miller and A. K. Hoffmann, *J. Am. Chem. Soc.*, **89**, 593 (1967).
(44) H. Budzikiewicz, C. Djerassi, and D. H. Williams, "Mass Spectrometry of Organic Compounds", Holden-Day, San Francisco, Calif., 1967, p 429.
(45) (a) J. L. Imbach, J. L. Barascut, B. L. Kam, and C. Tapiero, *Tetrahedron Lett.*, 129 (1974); (b) B. Rayner, C. Tapiero, and J. L. Imbach, *J. Heterocycl. Chem.*, **10**, 417 (1973); (c) J. L. Imbach and B. L. Kam, *J. Carbohydr., Nucleosides, Nucleotides*, **1**, 271 (1974).
(46) Y. Mizuno and T. Sasaki, *J. Am. Chem. Soc.*, **88**, 863 (1966).
(47) (a) J. A. Montgomery, K. Hewson, S. J. Clayton, and H. J. Thomas, *J. Org. Chem.*, **31**, 2202 (1966); (b) J. T. Witkowski, G. R. Kreishman, M. P. Schweizer, and R. K. Robins, *ibid.*, **38**, 180 (1973); (c) K. Anzai and M. Matsui, *Agric. Biol. Chem.*, **37**, 301, 2431 (1973).
(48) R. N. Hammer and J. Kleinberg, *Inorg. Synth.*, **4**, 12 (1953).
(49) The use of Linde AW-500 sieve or a mixture of both types led to very similar results.
(50) The alkaline spectrum must be taken rapidly. In 0.01 N sodium hydroxide the sample rapidly degraded to adenine.

Topography of Cyclodextrin Inclusion Complexes. 8. Crystal and Molecular Structure of the α-Cyclodextrin–Methanol–Pentahydrate Complex. Disorder in a Hydrophobic Cage[1]

B. Hingerty and W. Saenger*

Contribution from the Max-Planck-Institut für Experimentelle Medizin, Abteilung Chemie, Göttingen, Germany. *Received July 8, 1975*

Abstract: α-Cyclodextrin (α-CD, cyclohexaamylose) is a cyclic molecule consisting of six glucoses. Owing to its 5 Å wide annular aperture it is able to form inclusion complexes with small molecules even in aqueous solution. Complex formation was recently attributed to a strained, high-energy "tense" conformation of "empty" α-CD which transforms into an unstrained low-energy, "relaxed" conformation upon inclusion of some substrate molecule. In order to test this mechanism, the structure of the α-CD–methanol complex was of interest to study. The α-CD–methanol–pentahydrate adduct crystallizes in space group $P2_12_12_1$ with $a = 14.339$ Å, $b = 37.365$ Å, $c = 9.465$ Å. A total of 4283 intensities were collected using a diffractometer and corrected for absorption with empirical methods. The structure was solved on the basis of the isomorphous α-CD–propanol–4.8hydrate complex and refined by full-matrix least-squares methods to $R = 4.3\%$ for all the data. All hydrogen atoms except those of disordered hydroxyl groups were located from difference Fourier maps. The α-CD molecule is in an unstrained "relaxed" conformation stabilized through a ring of O(2)···O(3) hydrogen bonds between adjacent glucoses; all C(6)–O(6) bonds point away from the center of the molecule but one is twofold disordered, with 25% occupancy toward the center and in hydrogen bonding contact with the included methanol molecule. The latter assumes two sites at equal population: 50% near the O(6) side of the α-CD molecule and hydrogen bonded to the disordered O(6) group; the other 50% within the cavity of the α-CD and not involved in hydrogen bonding. Both methanol molecules perform excessive thermal motion and are held mainly by van der Waals contacts to C(3)–H and C(5)–H methine protons. This study clearly supports the mechanism of inclusion formation described at the outset.

Cyclodextrins (cycloamyloses) are cyclic molecules consisting of six to nine glucose units linked together through α(1 → 4) glucosidic bonds. The smallest of these molecules, α-cyclodextrin (α-CD), has an annular space 5 Å in diameter and assumes the shape of a truncated cone about 9 Å high, 8 Å at the narrow side, and 13.5 Å at the wide side. The narrow side is lined with the primary O(6) hydroxyl groups while the wide side is lined with secondary O(2) and O(3) hydroxyls. α-CD is therefore mainly hydrophilic in character at the outside of the cavity but at the inside it is hydrophobic, as here the lining consists of hydrogen atoms and of the O(4) atoms which link the glucose units.

α-CD is soluble in water and, owing to its cavity, it is able to form inclusion complexes with other guest (substrate) molecules even in aqueous solution.[2-5] A number of these complexes could be crystallized and were analyzed in detail by x-ray structural studies.[5] It was striking to find that in the water inclusion complex α-CD·$2H_2O$, which represents the "empty" α-CD molecule occurring in aqueous solution,[6] the α-CD appears unsymmetrical, collapsed to fit its 5 Å wide cavity to the 3.8 Å thick water molecules. In all the other complexes, even with krypton of 4 Å diameter,[7] the α-CD molecule is almost cyclic and the cavity is opened fully to accommodate the substrate molecules. On the basis of these results the following mechanism for the inclusion process was proposed.[5-7] In the "empty", collapsed state with two included water molecules, the α-CD is in a high-energy conformation owing to unfavorable conformational angles and to disruption of the otherwise fully developed ring of six hydrogen bonds between the O(2), O(3) hydroxyls of adjacent glucoses. When inclusion occurs with some substrate molecule, the α-CD assumes a circular, unstrained conformation with the formation of O(2)···O(3) hydrogen bonds. This represents a low-energy state (Figure 1). As there is an analogy between this mechanism and the mechanism proposed for the enzyme–substrate adduct formation,[8] the two states of the α-CD molecule have been termed "tense" and "relaxed".[5,7] The driving force toward inclusion complex formation resides mainly in the α-CD molecule itself while substrate–α-CD interactions are of minor importance. This mechanism also explains why a variety of substrates, ranging from the purely hydrophilic such as salts, methanol, and water to the purely hydrophobic such as halogen, krypton, and paraffins, react with α-CD. The conformational change of α-CD upon complex formation has also been observed by CD spectroscopy in aqueous solution.[9] The x-ray studies support this finding and provide a basis to understand