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Synthesis and antileukemic activity of certain D-fucopyranosyl nucleosides

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ABSTRACT

Based upon previously discovered antileukemic properties of 9-β-D-fucopyranosyladenine (**1**) in cell culture, four new nucleosides containing naturally occurring bases have been prepared from D-fucose. α-D-Fucopyranose tetraacetate was condensed with the silylated bases in either acetonitrile or 1,2-dichloroethane with tin(IV) chloride as the catalyst. The intermediate blocked nucleosides were obtained in crystalline form and deacetylated with methanolic sodium methoxide. 1-β-D-Fucopyranosyluracil (**8**), 1-β-D-fucopyranosylthymine (**9**), 1-β-D-fucopyranosylcytosine (**10**) as the hydrochloride salt, and 7-β-D-fucopyranosylguanine (**11**) were crystallized, and their structures were verified by spectroscopic techniques. Nucleosides **8** and **9** had only borderline activity against leukemia L1210 cells grown in culture, whereas nucleoside **11** had activity equal to **1**. However, nucleoside **10** proved to be twice as active as either **1** or **11**. The antileukemic activity, which was due to the inhibition of cell division, was reversible by transfer of the arrested cells to fresh media or by the addition of cytidine.

INTRODUCTION

The synthesis of nucleosides derived from hexopyranoses is generally straightforward, since their preparation does not require any special techniques or conversion to unique derivatives in order to obtain the desired compounds. Once the hexoses are available, either commercially or by synthesis, the sugars are acetylated or benzoylated and then coupled to suitable derivatives of nitrogenous bases. Indeed, the very first nucleoside ever synthesized that contained a natural base was in the hexopyranose form, namely 9-β-D-glucopyranosyladenine[1]. Since that time, a number of nucleosides have been prepared from hexoses in the pyranose ring form. Unfortunately, during the search for nucleosides having biological activity, nucleoside analogues derived from hexopyranoses have not fared too well. This may be due not only to a carbon chain having one too many

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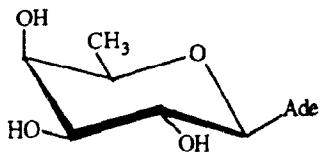
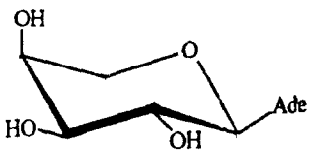
carbon atoms, but also to a ring size that is too large and whose stereochemistry is very different from that of the naturally occurring D-ribofuranose or 2-deoxy-D-ribofuranose moieties. Nevertheless, even among the hexopyranose nucleosides there are some compounds of biological interest. One of these is 1-(2-deoxy-β-D-*arabino*-hexopyranosyl)thymine[2], which was recognized many years ago to be an inhibitor of a pyrimidine nucleoside phosphorylase from Ehrlich ascites tumor[3]. The related pentopyranosyl analogue, 1-(2-deoxy-β-D-*threo*-pentopyranosyl)thymine, is also known to inhibit the same enzyme[4]. A number of ketohexopyranosyl nucleosides[5,6] and α,β-unsaturated ketohexopyranosyl nucleosides[7] were found to have antitumor activity in vivo as well as in vitro. Recently, there has been a surge of research activity to prepare some hexopyranosyl nucleoside analogues as potential anti-HIV agents[8–10], but none of these unusual compounds possessed antiviral activity.

Several years ago we reported on antiproliferative activity of 9-β-D-fucopyranosyladenine [9-(6-deoxy-β-D-galactopyranosyl)adenine] (**1**) against leukemia



1



2

Ade = adenine

L1210 cells in culture[11]. A number of 6-deoxyhexopyranosyl adenine nucleoside analogues of **1** were synthesized, but none of these had the activity exhibited by **1**. Only the structurally related pentopyranose nucleoside, 9-α-L-arabinopyranosyladenine (**2**) had activity, which was significantly weaker than **1**. This possibly indicated a preference for the methyl group rather than an hydrogen atom at the 5′-position in addition to the D-galacto configuration. In the present paper, we report the synthesis and in vitro growth inhibitory activity against leukemia L1210 cells of some D-fucopyranosyl nucleosides containing the naturally occurring bases, uracil, thymine, cytosine, and guanine. The adenine nucleoside **1** was also prepared again by a different route to serve as a standard against which the new nucleosides could be compared in the antileukemic studies. Moreover, previous preparations of **1** used mercuric salts of 6-benzamidopurine[11,12], so it appeared worthwhile to repeat the preparation by a route that did not utilize mercury, in order to exclude the possibility that the previously observed growth inhibition was due to metal contamination of the nucleoside. The synthetic route reported here for **1** has a number of chemical advantages as well because it bypasses the formation of the blocked nucleoside picrate and the regeneration and crystalliza-

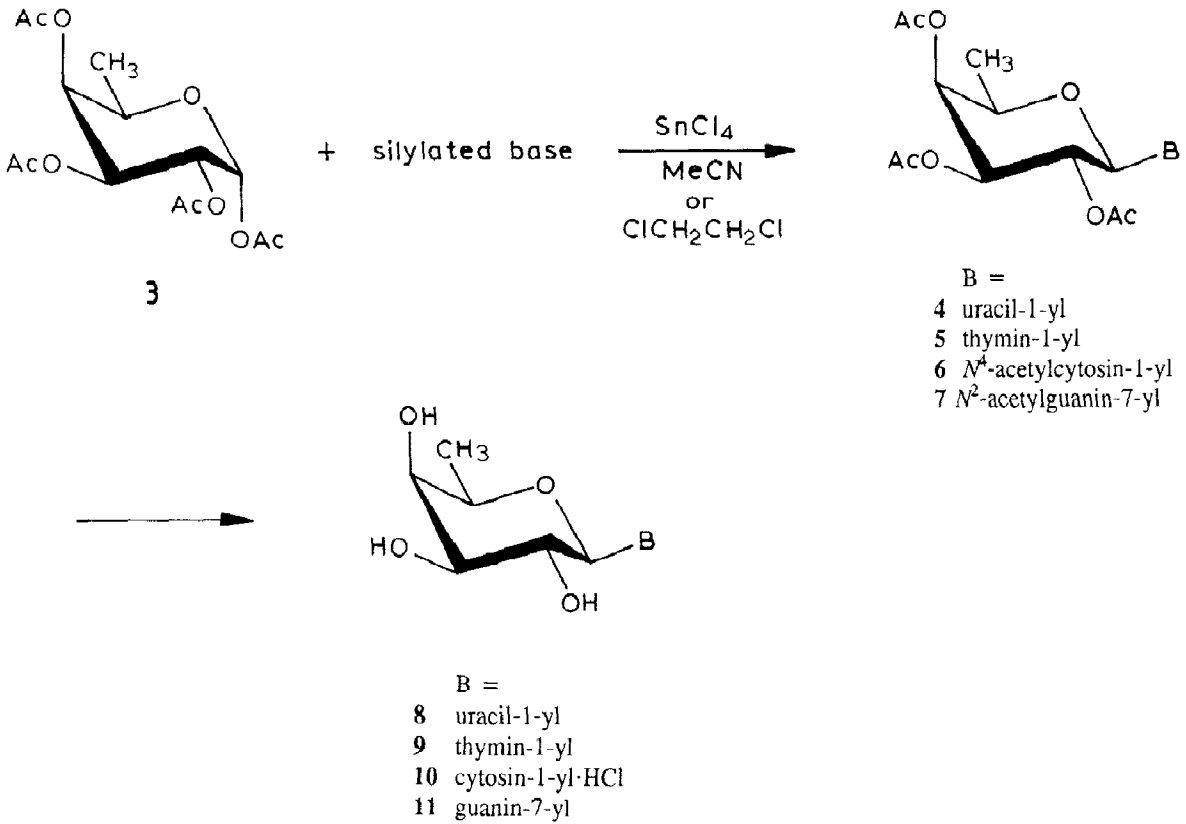
tion of the blocked nucleoside[12]. The overall yield of **1** by this new, shorter, and less tedious route was approximately the same as by the previous route.

RESULTS AND DISCUSSION

Chemistry.—The procedure for coupling α-D-fucopyranose tetraacetate (**3**, Scheme 1) with the bases was the one developed by Niedballa and Vorbrüggen[13] for pyrimidines and applied by other workers to purines[14]. The choice of reaction solvent for the uracil and thymine couplings was dictated by the earlier studies[13,15], which demonstrated that in 1,2-dichloroethane pyranoses gave significant amounts of N-3 substituted products in addition to the desired N-1 products. Therefore, acetonitrile was used to reduce the amount of any N-3 substitution, although no reactions were actually run in 1,2-dichloroethane to determine the ratio of N-1 to N-3 products. The coupling reactions for the other bases were performed in 1,2-dichloroethane. The yields of crystalline blocked pyrimidine nucleosides **4**, **5**, and **6** were all above 90%. The yield of the blocked guanine nucleoside **7** was only moderate (52%), and as will be shown below, the linkage of the sugar is to the N-7 position rather than to N-9. Attempts to isolate the N-9 substituted product from the mother liquors were discouraged by the large number of unidentified products as revealed by TLC. Attempts to prepare the N-9 substituted nucleoside using the techniques advocated by Garner and Ramakanth[16] did not meet with success either, also due to an intractable mixture of products.

The acetyl groups of the nucleosides **4–7** were removed in methanolic sodium methoxide, and the free nucleosides **8–11** were obtained in excellent yields. One of these, 1-β-D-fucopyranosylcytosine was obtained as a noncrystalline solid, but did not give an acceptable elemental analysis. Further attempts at purification were not successful; therefore, this compound was synthesized again and converted to the hydrochloride salt (**10**), which crystallized easily.

The structures of the new nucleosides were confirmed by elemental analysis and spectroscopy. The UV spectra supported the linkage of the D-fucose moiety to N-1 of the pyrimidine rings[17] and provided the first indication that the linkage to guanine was at N-7 rather than at N-9 in compound **11**. The latter was based upon the absorption maxima near 285 nm in solutions of neutral and basic pH, and the shift to the shorter 250–260 nm region in a solution of acidic pH[16,18]. Guanine nucleosides containing the N-9 linkage, such as guanosine, generally exhibit absorption maxima that are at shorter wavelengths (~ 265 nm) at neutral and basic pH, and these maxima will also shift to a somewhat shorter wavelength (250–260 nm) in acidic solutions[19]. The N-7 linkage also appeared to be supported by the ^{1}H NMR spectrum. Nucleoside **11** had a singlet peak at δ 8.16 for H-8 of the guanine ring. The H-8 resonance for the guanine moiety of nucleosides generally occurs well below δ 8.00 for those compounds substituted at N-9; however, those nucleosides substituted at N-7 generally have this peak well above δ 8.00 (refs 16 and 18).


AcO
CH3
O
AcO
AcO
OAc
3
+ silylated base
SnCl4
MeCN
or
ClCH2CH2Cl
AcO
CH3
O
AcO
B
OAc
B =
4 uracil-1-yl
5 thymin-1-yl
6 N4-acetylcytosin-1-yl
7 N2-acetylguanin-7-yl
OH
CH3
O
HO
B
OH
B =
8 uracil-1-yl
9 thymin-1-yl
10 cytosin-1-yl·HCl
11 guanin-7-yl

Scheme 1.

The ^{1}H NMR spectra of nucleosides **4–11** completely supported the structural assignments made here. Of prime importance was the coupling constant $J_{1',2'}$ of ~ 9 Hz at the H-1′ peak, which demonstrated the *trans*-diaxial orientation of the protons at C-1 and C-2. Another *trans*-diaxial orientation was found between the protons on C-2′ and C-3′, as shown by the $J_{2',3'}$ values of ~ 10 Hz. These results clearly show that these nucleosides have the β configuration at the anomeric carbon atom, and that the D-fucose moiety is in the $^4C_1$ conformation[20]. It had been expected that the main product of these reactions would be in the β configuration due to the directive effect of the acetyl groups at C-2 of the sugar.

Antiproliferative activity.—The new nucleosides **8–11** were tested for antiproliferate activity against leukemia L1210 cells grown in culture. The results are shown in Table I and are compared to the results obtained for **1**.

The antileukemic activity of the new preparation of **1** was exactly the same as previously reported, showing that the earlier results were not due to contamination by mercury. The uracil and thymine nucleosides (**8** and **9**) had only borderline activity, whereas the guanine nucleoside **11** exhibited antileukemic activity equal to that of nucleoside **1**. However, the cytosine nucleoside **10** was twice as active as nucleosides **1** or **11**. In our earlier paper[11], we had shown that only the D-fucopyranose structure conferred significant activity when adenine was the base. The present results appear to further support a unique role for D-fucose in the pyranose ring form. We have already demonstrated that neither product of the hydrolysis of **1**, D-fucose nor adenine, caused growth inhibition of L1210 cells[11]. Similarly, cytosine and guanine, the parent bases for nucleosides **10** and **11**, respectively, were tested and found not to cause inhibition of growth.

In an experiment to determine the reversibility of the inhibition caused by **10**, the cells were grown for 48 h in a medium containing a 30 μM solution of the nucleoside, which in this experiment gave 65% inhibition. The surviving cells were washed three times with Krebs solution and reincubated in fresh media. For the first 24 h, the growth of these cells was somewhat sluggish compared to the control, but after 24 h, they displayed the same growth kinetics as the control cells. In a different reversal experiment, cytidine (100 μM), added at the same time as nucleoside **10**, was able to reverse the inhibition by 84%. These data suggest that growth inhibition induced by **10** is competitive and involves, at least in part, the inhibition of an enzyme involved in nucleoside metabolism or transport.

EXPERIMENTAL

General methods.—Elemental analyses were performed by Spang Microanalytical Laboratory, Eagle Harbor, MI. Melting points were obtained on a Kofler hot-stage and are corrected values. Optical rotations were measured with a Perkin–Elmer Model 141 polarimeter, and UV spectra were recorded on a Beckman Model 25 spectrophotometer. NMR spectra were obtained on a Bruker 250 MHz instrument at a probe temperature of 25°C with Me_4Si as the inter-

nal reference in $CDCl_3$ and 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) in Me_2SO-d_6.

Paper chromatography was performed by a descending technique on Whatman No. 1 paper using adenine as a reference. UV-absorbing spots were located with a Mineralight lamp (254 nm). Mobilities are reported as R_{ade} values, defined as the ratio of the distance the nucleoside migrated to the distance the reference adenine migrated. The solvent systems used were (*A*) 5% aq Na_2HPO_4 and (*B*) 5:2:3 1-butanol–AcOH–H_2O.

Materials.—D-Fucose was purchased from Pfanstiehl Laboratories and acetylated as previously described to give crystalline α-D-fucopyranose tetraacetate (**3**) (ref 12). Uracil, cytosine, adenine, and N^2-acetylguanine were obtained from Sigma Chemical Company, and thymine was obtained from Aldrich Chemical Company. N^4-Acetylcytosine was prepared as described by Coddington and Fox[21], and N^6-benzoyladenine was prepared as described by Kohn et al.[22].

General procedures for the synthesis of pyrimidine nucleosides.—The procedures described by Niedballa and Vorbrüggen[13,15] were used. The bases (uracil, thymine, N^4-acetylcytosine) were converted to their trimethylsilyl derivatives and used without distillation. The silylated bases were reacted with α-D-fucopyranose tetraacetate (**3**) at room temperature in the presence of $SnCl_4$ as catalyst. The uracil and thymine nucleoside preparations were performed in MeCN, and the cytosine nucleoside was prepared in 1,2-dichloroethane. After the usual workup[13,15], the blocked nucleosides were crystallized. The acetyl groups were removed in solutions of methanolic NaOMe at room temperature and brought to neutrality with Amberlite CG-120 (H^+) ion-exchange resin. Filtration and evaporation afforded the free nucleosides.

1-(2,3,4-Tri-O-acetyl-β-D-fucopyranosyl)uracil (**4**).—Crystallization from EtOH in three crops gave a total yield of 95%. Recrystallization from EtOH gave a product in the form of long prismatic-shaped crystals; mp 140–143°C to a very viscous syrup exhibiting birefringence under the polarizing lenses of the microscope; $[\alpha]_D^{22}$ +15.2° (*c* 1.10, $CHCl_3$); UV_{max} (EtOH) 257, 211 nm; UV_{min} (EtOH) 227 nm; ^1H NMR ($CDCl_3$) δ 9.00 (br s, 1 H, NH), 7.42 (d, 1 H, $J_{6,5}$ = 8.0 Hz, H-6), 5.84 (d, 1 H, $J_{5,6}$ 8.0 Hz, H-5), 5.81 (d, 1 H, $J_{1',2'}$ 8.3 Hz, H-1'), 5.35–5.18 (complex overlapping m, 3 H, H-2',3',4'), 4.06 (q, 1 H, $J_{5',6'}$ 6.4 Hz, H-5'), 2.22, 2.02, 2.00 (all s, 3 H each, OAc), 1.20 (d, 3 H, H-6'). Anal. Calcd for $C_{16}H_{20}N_2O_9$: C, 50.00; H, 5.25, N, 7.29. Found: C, 50.38; H, 5.55; N, 6.98.

1-β-D-Fucopyranosyluracil (**8**).—A white solid residue was obtained, which was recrystallized from EtOH–H_2O. The yield was 81%; mp 270–271°C (dec); $[\alpha]_D^{27}$ +40.3° (*c* 0.591, H_2O); UV_{max} (pH 1) 259 nm (ε 9740), (H_2O) 259 (ε 9025), (pH 13) 258 (ε 7255); UV_{min} (pH 1) 239 nm (ε 2305), (H_2O) 240 (ε 1530), (pH 13) 243 (ε 6200); ^1H NMR (Me_2SO-d_6) δ 11.31 (br s, 1 H, NH), 7.65 (d, 1 H, $J_{6,5}$ = 8.1 Hz, H-6), 5.70 (d, 1 H, $J_{5,6}$ 8.1 Hz, H-5), 5.33 (d, 1 H, $J_{1',2'}$ 9.0 Hz, H-1'), 5.25 (d, 1 H, OH), 4.95 (s, 1 H, OH), 4.59 (d, 1 H, H-4'), 3.79 (q, 1 H, $J_{5',6'}$ 6.3 Hz, H-5'), 3.66 (m, 1 H, H-2'), 3.51 (m, 1 H, H-3'), 3.42 (s, 1 H, OH), 1.15 (d, 3 H, H-6'); R_{ade} 2.23

(*A*), 0.84 (*B*). Anal. Calcd for $C_{10}H_{14}N_2O_6$: C, 46.51; H, 5.46; N, 10.85. Found: C, 46.67; H, 5.35; N, 10.99.

*1-(2,3,4-Tri-*O*-acetyl-β-*D*-fucopyranosyl)thymine* (**5**).—The nucleoside was crystallized from cold EtOH in two crops (98% yield) as clear platelets; mp 129–132°C to a viscous liquid; $[\alpha]_D^{22}$ −2.0° (*c* 1.02, $CHCl_3$); UV_{max} (EtOH) 261, 213 nm; UV_{min} (EtOH) 231 nm; ^1H NMR ($CDCl_3$) δ 9.32 (br s, 1 H, NH), 7.19 (s, 1 H, H-6), 5.80 (d, 1 H, $J_{1',2'}$ 9.4 Hz, H-1′), 5.32 (d, 1 H, H-4′), 5.26 (m, 1 H, $J_{2',3'}$ 10.2 Hz, H-2′), 5.19 (m, 1 H, $J_{3',4'}$ 3.2 Hz, H-3′), 4.05 (q, 1 H, $J_{5',6'}$ 6.4 Hz, H-5′), 2.21 (s, 3 H, OAc), 1.96 (overlapping s, 4 H, OAc and thymine CH_3), 1.94 (s, 3 H, OAc), 1.21 (d, 3 H, H-6′), Anal. Calcd for $C_{17}H_{22}N_2O_9$: C, 51.25; H, 5.57; N, 7.03. Found: C, 51.32, H, 5.66; N, 6.81.

*1-β-*D*-Fucopyranosylthymine* (**9**).—A white foam was obtained, which was dissolved in warm EtOH containing a few drops of H_2O. Addition of a few drops of Et_2O brought the solution to turbidity, and it was stored in the refrigerator overnight. The resulting crystalline mass was broken up, filtered off, and washed with a mixture of EtOH and Et_2O. A second crop was obtained by concentrating the mother liquor and adding Et_2O to turbidity. The total yield was 94%; mp 228–229°C, with softening and a change in form between 151–160°C; $[\alpha]_D^{27}$ +33.0° (*c* 0.679, H_2O); UV_{max} (pH 1) 265 nm (ε 8600), (H_2O) 265 (ε 8600), (pH 13) 266 (ε 6540); UV_{min} (pH 1) 234 nm (ε 2160), (H_2O) 233 (ε 2140), (pH 13) 247 (ε 4995); ^1H NMR (Me_2SO-d_6) δ 11.30 (br s, 1 H, NH), 7.53 (s, 1 H, H-6), 5.31 (d, 1 H, $J_{1',2'}$ 9.2 Hz, H-1′), 5.20 (d, 1 H, OH), 4.92 (s, 1 H, OH), 4.58 (d, 1 H, H-4′), 3.76 (q, 1 H, $J_{5',6'}$ 6.3 Hz, H-5′), 3.67 (m, 1 H, H-2′), 3.50 (m, 1 H, H-3′), 3.40 (s, 1 H, OH), 1.82 (s, 3 H, thymine CH_3), 1.15 (d, 3 H, H-6′). R_{ade} 2.48 (*A*), 0.97 (*B*). Anal. Calcd for $C_{11}H_{16}N_2O_6 \cdot H_2O$: C, 45.51; H, 6.25; N, 9.65. Found: C, 45.15; H, 6.26; N, 9.29.

N^4-*Acetyl-1-(2,3,4-tri-*O*-acetyl-β-*D*-fucopyranosyl)cytosine* (**6**).—This nucleoside formed flowery clusters of tiny white needles from cold EtOH–hexane. The yield was 94% in three crops; mp 247–248°C with tiny droplets forming on the cover slip above 135°C; $[\alpha]_D^{22}$ +80.0° (*c* 1.10, $CHCl_3$); UV_{max} (EtOH) 300, 251, 214 nm; UV_{min} (EtOH) 277, 227 nm; ^1H NMR ($CDCl_3$) δ 9.77 (br s, 1 H, NHAc), 7.79 (d, 1 H, $J_{5,6}$ 7.6 Hz, H-5), 7.51 (d, 1 H, $J_{6,5}$ 7.6 Hz, H-6), 6.02 (d, 1 H, $J_{1',2'}$ 9.0 Hz, H-1′), 5.35–5.18 (complex overlapping m, 3 H, H-2′,3′,4′), 4.06 (q, 1 H, $J_{5',6'}$ 6.4 Hz, H-5′), 2.26 (s, 3 H, NHAc), 2.21, 1.98, 1.95 (all s, 3 H each, OAc), 1.21 (d, 3 H, H-6′). Anal. Calcd for $C_{18}H_{23}N_3O_9$: C, 50.82; H, 5.45; N, 9.88. Found: C, 50.84; H, 5.31; N, 9.74.

*1-β-*D*-Fucopyranosylcytosine hydrochloride* (**10**).—The residue was dissolved in 1.5 M HCl. A small amount of absolute EtOH was added, and the mixture was evaporated under reduced pressure (30°C) to dryness. A few additional drops of HCl were added, and the EtOH addition and evaporation was repeated. The white solid that remained was triturated with cold EtOH, filtered by suction, and washed with cold EtOH, then with Et_2O. The product was recrystallized by dissolution in a minimum amount of H_2O, followed by addition of EtOH and chilling in an ice bath. Three crops of clear, rectangular platelets (91% yield) were obtained, which

upon heating slowly frosted over above 150°C; mp 175–178°C with slow decomposition above 181°C; $[\alpha]_D^{26}$ +41.7° (*c* 1.03, H_2O); UV_{max} (pH 1) 277 nm (ε 12410), (H_2O) 271 (ε 8835), 269, 238 sh (ε 8035); UV_{min} (pH 1) 242 nm (ε 2145), (H_2O) 248 (ε 6345), (pH 13) 254 (ε 7410); ^1H NMR (Me_2SO-d_6) δ 8.02 (d, 1 H, $J_{6,5}$ 7.8 Hz, H-6), 6.29 (d, 1 H, $J_{5,6}$ 7.8 Hz, H-5), 5.38 (d, 1 H, $J_{1',2'}$ 8.8 Hz, H-1′), 3.86 (q, 1 H, $J_{5',6'}$ 6.3 Hz, H-5′), 3.7–3.6 (complex overlapping m, 3 H, H-2′,3′,4′), 1.17 (d, 3 H, H-6′); R_{ade} 2.28 (*A*), 0.74 (*B*). Anal. Calcd for $C_{10}H_{15}N_3O_5 \cdot HCl$: C, 40.89; H, 5.49; N, 14.31. Found: C, 40.96; H, 5.42; N, 14.28.

General procedure for the synthesis of purine nucleosides.—These procedures followed, in general, the application of the pyrimidine nucleoside synthesis to purines[14]. The bases (N^2-acetylguanine and N^6-benzoyladenine) were converted to their trimethylsilyl derivatives and used without distillation. The coupling reactions of the bases to the sugar were performed in a manner similar to the pyrimidine nucleosides, only the solutions had to be heated under reflux. The guanine nucleoside synthesis was performed in MeCN (6 h reaction time), and the adenine nucleoside was prepared in 1,2-dichloroethane (2 h reaction time). The blocking groups of the adenine nucleoside were removed as described above for the pyrimidine nucleosides, whereas the guanine nucleoside was heated under reflux for 2 h. The latter solution was neutralized with glacial acetic acid rather than with the ion-exchange resin.

N^2-*Acetyl-7-(2,3,4-tri-*O*-acetyl-β-*D*-fucopyranosyl)guanine* (**7**).—Crystallization was effected from EtOH (52% yield), and recrystallization from MeOH gave small, prismatic crystals; mp 297–299°C; $[\alpha]_D^{23}$ +4.4° (*c* 0.909, Me_2SO); UV_{max} (MeOH) 282 sh, 264, 224 nm; UV_{min} (MeOH) 234 nm; ^1H NMR (Me_2SO-d_6) δ 12.21, 11.65 (both br s, 1 H each, NH and NHAc), 8.41 (s, 1 H, H-8), 6.12 (d, 1 H, $J_{1',2'}$ 9.0 Hz, H-1′), 5.75 (br unresolved, 1 H, $J_{2',3'}$ 10.0 Hz, H-2′), 5.43 (dd, 1 H, $J_{3',4'}$ 3.1 Hz, H-3′), 5.24 (d, 1 H, H-4′), 4.38 (q, 1 H, $J_{5',6'}$ 6.4, H-5′), 2.24, 2.19, 1.97, 1.81 (all s, 3 H each, NHAc and OAc), 1.10 (d, 3 H, H-6′). Anal. Calcd for $C_{19}H_{23}N_5O_9$: C, 49.03; H, 4.98; N, 15.05. Found: C, 49.03; H, 5.04; N, 14.94.

7-β-D-*Fucopyranosylguanine* (**11**).—Crystallization was effected from water (86% in two crops). This nucleoside did not melt below 360°C, but it did slowly decompose at temperatures above 181°C; $[\alpha]_D^{27}$ +3.8° (*c* 0.342, 0.1 N NaOH); UV_{max} (pH 1) 252 nm (ε 3740), (H_2O) 287 (ε 3685), 215 (ε 9110), (pH 13) 282 (ε 3370), 215 (ε 22020); UV_{min} (pH 1) 233 (ε 3430), (H_2O) 260 (ε 1370), (pH 13) 257 (ε 1630); ^1H NMR (Me_2SO-d_6) δ 11.12 (br s, 1 H, NH), 8.16 (s, 1 H, H-8), 6.38 (br s, 2 H, NH_2), 5.39 (d, 1 H, $J_{1',2'}$ 8.9 Hz, H-1′), 5.32 (d, 1 H, OH), 4.99 (s, 1 H, OH), 4.57 (d, 1 H, H-4′), 4.02 (m, 1 H, H-2′), 3.80 (q, 1 H, $J_{5',6'}$ 6.3, H-5′), 3.50 (m, 1 H, H-3′), 3.44 (s, 1 H, OH), 1.16 (d, 3 H, H-6′). R_{ade} 2.41 (*A*), 0.75 (*B*). Anal. Calcd for $C_{11}H_{15}N_5O_5 \cdot H_2O$: C, 41.90; H, 5.44; N, 22.21. Found: C, 42.07; H, 4.81; N, 22.31.

9-β-D-*Fucopyranosyladenine* (**1**).—The nucleoside was obtained in an overall yield from **3** of 41%. This compound was identical in every way to the previously prepared nucleoside; mp 170°C with a slow change in state starting at about 110°C;

TABLE I

Inhibitory activity of D-fucopyranosyl nucleosides against leukemia L1210 cells grown in culture

Nucleoside	Molar concentration (μM) for 50% inhibition [a,b]
1	60 ± 0.3
8	> 100
9	> 100
10	30 ± 0.2
11	60 ± 0.5

[a] Inhibitory results were calculated from log–dose response curves. [b] The standard deviations were calculated from the results from 2–3 separate experiments, each one done in duplicate.

$[\alpha]_D^{23}$ +10.5° (*c* 1.88, H_2O). Lit.[12] mp 170°C with a similar change taking place at 106–110°C; $[\alpha]_D^{26}$ +10.9°C (*c* 1.88, H_2O). The IR spectra of the various preparations were identical. R_{ade} 1.62 (A), 0.84 (B).

Antileukemic assay.—Details concerning the reagents and methods used for the L1210 growth inhibitory studies are reported in the earlier paper dealing with the sugar analogs of **1** (ref 11). In the present studies, the initial cell density was 2×10^5/mL. The incubation was allowed to proceed for 48 h at 37°C in a 95:5% O_2–CO_2 humidified atmosphere. The cell number and viability was determined with an hemocytometer. No dead cells or cell fragments were detected by staining (Trypan Blue) and light microscopy. In experiments with the cytosine nucleoside **10**, growth of the arrested cells could be stimulated again by transfer of the cells to fresh media, or by addition of cytidine.

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REFERENCES

1 E. Fischer and B. Helferich, *Ber.* 47 (1914) 210–235.
2 W.W. Zorbach and G.J. Durr, *J. Org. Chem.*, 27 (1962) 1474–1477.
3 P. Langen and G. Etzold, *Biochem. Z.*, 339 (1963) 190–197.
4 G. Etzold, B. Preussel, and P. Langen, *Mol. Pharmacol.*, 4 (1968) 20–24.
5 K. Antonakis and I. Chouroulinkov, *Biochem. Pharmacol.*, 23 (1974) 2095–2100.
6 K. Antonakis, *Chimia*, 29 (1975) 59–62.
7 K. Antonakis, T. Halmos, J. Bach, and I. Chouroulinkov, *Eur. J. Med. Chem.*, 15 (1980) 237–240.
8 P. Herdewijn and A. Aerschot, *Bull. Soc. Chim. Belg.*, 99 (1990) 895–901.
9 Z. Kaluza, E.B. Pedersen, C.M. Nielsen, and M. Chmielewski, *Acta Chem. Scand.*, 44 (1990) 294–296.
10 P. Hansen, J. Lan, E.B. Pedersen, and C.M. Nielsen, *J. Liebigs Ann.*, (1990) 1079–1082.
11 L.M. Lerner, B. Sheid, and E. Gaetjens, *J. Med. Chem.*, 30 (1987) 1521–1525.
12 L.M. Lerner, *Carbohydr. Res.*, 19 (1971) 255–258.
13 U. Niedballa and H. Vorbrüggen, *J. Org. Chem.*, 39 (1974) 3654–3660.
14 F.W. Lichtenthaler, P. Voss, and A. Heerd, *Tetrahedron Lett.*, (1974) 2141–2144.

15 U. Nieballa and H. Vorbrüggen, *J. Org. Chem.*, 41 (1976) 2084–2086.
16 P. Garner and S. Ramakanth, *J. Org. Chem.*, 53 (1988) 1294–1298.
17 J.J. Fox and I. Wempen, *Adv. Carbohydr. Chem.*, 14 (1959) 283–380.
18 N.E. Poopeiki, E.I. Kvasyuk, I.A. Mikhailopulo, and M.J. Lidaks, *Synthesis*, (1985) 605–609.
19 G.H. Beaven, E. Holiday, and E.A. Johnson, in E. Chargaff, J.N. Davidson (Eds.), *The Nucleic Acids*, Vol. 1, Academic Press, New York, 1955, pp. 493–553.
20 L.B. Townsend, in W.W. Zorbach and R.S. Tipson (Eds.), *Synthetic Procedures in Nucleic Acid Chemistry*, Vol. 2, Interscience Publishers, New York, 1973, pp. 267–398.
21 J.F. Codington and J.J. Fox, *Methods Carbohydr. Chem.*, 2 (1963) 113–116.
22 P. Kohn, R. Samaritano, and L.M. Lerner, in W.W. Zorbach and R.S. Tipson (Eds.), *Synthetic Procedures in Nucleic Acid Chemistry*, Vol. 1, Interscience Publishers, New York, 1968, pp. 117–122.