tractions established that equilibrium had been achieved under these conditions.

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Reactions of 2-Acyloxyisobutyryl Halides with Nucleosides. 6.[1a] Synthesis and Biological Evaluation of Some 3'-Acyl Derivatives of 2,2'-Anhydro-1-(β-D-arabinofuranosyl)cytosine Hydrochloride[1b]

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The reactions of cytidine with 22 different 2-*O*-acyloxyisobutyryl chlorides lead to the isolation of the corresponding 2,2'-anhydro-1-(3'-*O*-acyl-β-D-arabinofuranosyl)cytosine hydrochlorides **9**. These compounds, which all show cytotoxicity against HeLa cells in tissue culture, have been examined for antiviral and antileukemic activity. Activity against DNA viruses (vaccinia and Herpes) in tissue culture is maximal in compounds containing acyl groups with 8–12 carbon atoms. Activity against L1210 leukemia in mice varies markedly according to the length of the acyl groups, and high activities were observed in the case of long-chain (C_{16}–C_{22}) esters. The reaction between cytidine and *O*-acetylsalicyloyl chloride provides an alternate route for the synthesis of 3'-*O*-Ac cycloC hydrochloride.

Of the myriad of nucleoside derivatives that have been examined for potential antitumor activity, 1-(β-D-arabinofuranosyl)cytosine (araC) remains the one that has found widest clinical use, particularly in the therapy of acute leukemias.[3] AraC is, however, rapidly degraded to the biologically inactive 1-(β-D-arabinofuranosyl)uracil by the ubiquitous enzyme cytidine deaminase, the plasma half-life in man being only 12 min.[4] Since the drug exerts its cytotoxic effect only during the late S phase and early G_2 phase of the cell cycle,[5] this short half-life has necessitated the clinical use of complex dosage schedules or, in particular, continuous intravenous infusion.[3]

The pronounced antileukemic,[3] immunosuppressive,[6] and anti-DNA viral[7] activities of araC have stimulated the synthesis of a considerable number of derivatives of the parent compound. In general, the addition of substituents on the cytosine ring has led to compounds of reduced activity or, at best, to compounds showing no marked advantage over araC itself.[7b,8] Also, certain biologically irreversible modifications of the sugar moiety (e.g., methyl ethers)[9] lead to a loss of activity although more subtle changes such as the formation of 4'-thio[10] or 2'-halogenated[11] derivatives can be tolerated. Of particular interest have been the observations by the Upjohn group that various 5'-esters of araC, as well as certain 2'- and 3'-esters,[12e] show high biological activity of a long duration since these substances are not substrates for cytidine deaminase[12] and slowly release araC following enzymatic hydrolysis. Also, recent work from Japan has reported that 2,2'-anhydro-1-(β-D-arabinofuranosyl)cytosine hydrochloride (cycloC),[13] a substance which is itself resistant to cytidine deaminase[14] but which is slowly hydrolyzed to araC under physiological conditions, is a highly effective antitumor agent[15] with toxicity somewhat less than that

Table I. Representative 2-Acyloxyisobutyric Acids (7) and Acid Chlorides (8)

Acyl group	Formula	Mol wt	Analyses	Mp or bp (mm), °C	Ir, cm^{-1}
(1) Acids (7)					
Decanoyl	$C_{14}H_{26}O_4$	258.36	C, H	30.5-31	1720, 1740
Dodecanoyl	$C_{16}H_{30}O_4$	286.40	C, H	41-42	1720, 1740
Myristoyl	$C_{18}H_{34}O_4$	314.47	C, H	55-56	1720, 1740
Palmitoyl	$C_{20}H_{38}O_4$	342.52	C, H	55-57	1710, 1730
Stearoyl	$C_{22}H_{42}O_4$	370.58	C, H	60-61[a]	1715, 1740
Arachidyl	$C_{24}H_{46}O_4$	398.63	C, H	70-71	1725, 1745
Behenoyl	$C_{26}H_{50}O_4$	426.69	C, H	72-73	1720, 1735
Benzoyl	$C_{11}H_{12}O_4$	208.21	C, H	140-144	
Phenylacetyl	$C_{12}H_{14}O_4$	222.24	C, H	60-63	1715, 1760
(2) Acid chlorides (8)					
Decanoyl	$C_{14}H_{25}O_3Cl$	276.81	C, H	140 (0.1)	1745, 1805
Dodecanoyl	$C_{16}H_{29}O_3Cl$	304.87	C, H[b]	150 (0.1)	1745, 1805
Myristoyl	$C_{18}H_{33}O_3Cl$	332.92	C, H	175 (0.07)	1740, 1805
Palmitoyl	$C_{20}H_{37}O_3Cl$	360.97	C, H		1740, 1805
Stearoyl	$C_{22}H_{41}O_3Cl$	389.02	C, H	53-54	1745, 1805
Benzoyl	$C_{11}H_{11}O_3Cl$	226.66	C, H	102 (0.35)	

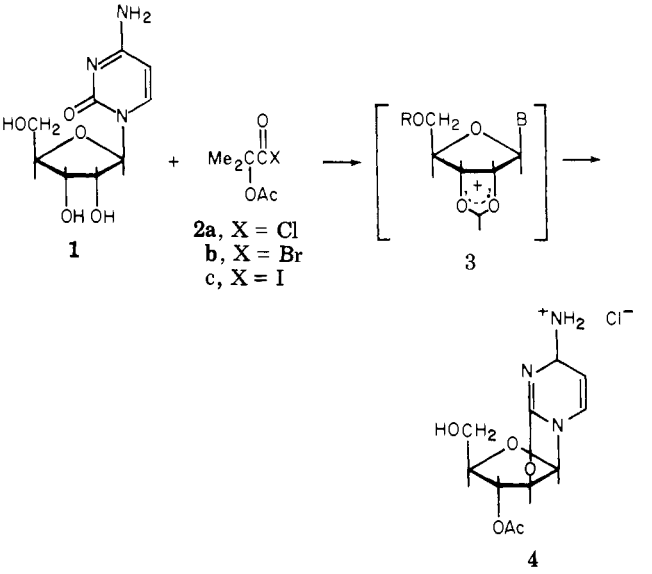
[a] Reported[23a] mp 59°. [b] Calcd: C, 63.04; H, 9.59. Found: C, 63.66; H, 10.17.

of araC.[16] This compound has been the subject of detailed pharmacological investigation[17] and clinical trial.[18] A number of analogues of cycloC substituted on the heterocyclic ring have been prepared for biological evaluation.[13d,19]

In recent years we have been engaged in a systematic study on the reactions of 2-acetoxyisobutyryl halides **2** with vicinal diols and, in particular, with ribonucleosides. This work has shown that the reaction of the 2',3'-diol function of a nucleoside with **2** leads initially to a 2',3'-acetoxonium ion **3** which then reacts further in one of several ways depending upon the nature of the nucleoside. Purine nucleosides lead principally to 2'-*O*-acetyl-3'-deoxy-3'-halo-β-D-xylofuranosyl nucleosides and to lesser amounts of their 3'-*O*-acetyl-2'-deoxy-2'-halo-β-D-arabinofuranosyl isomers.[20] Uridine and its analogues, however, lead to 3'-*O*-acetyl-2'-deoxy-2'-halo-β-D-ribofuranosyl nucleosides via an O^2,2'-anhydronucleoside arising from participation of the pyrimidine ring with the 2',3'-acetoxonium ion.[21] Of particular pertinence is the fact that cytidine (**1**), and variously modified cytidine analogues, reacted with **2a** to form stable 2,2'-anhydro-1-(3-*O*-acetyl-β-D-arabinofuranosyl)cytosine hydrochlorides (e.g., **4**),[13d] the 5'-hydroxyl function initially appearing as a readily cleaved 2,5,5-trimethyl-1,3-dioxolan-4-on-2-yl ether. By hydrolysis with acid or with base, **4** or its labile 5'-ether could be converted in high yield into either cycloC or araC, respectively, and this constitutes an efficient and economical synthesis of both compounds.

It was also shown that the above scheme could be extended to the preparation of homologous 3'-*O*-acyl cycloC's, the reaction of 2-butyryloxyisobutyryl chloride with cytidine readily giving the 3'-*O*-butyryl analogue of **4**.[13d] It was of interest to attempt to further extend this reaction so as to make available a larger series of 3'-*O*-acyl derivatives of cycloC for biological evaluation. As this work progressed it became apparent that the antiviral and antileukemic activities of these compounds were markedly affected by the precise nature of the 3'-*O*-acyl function. In this paper we describe the synthesis of a considerable number of such 3'-esters and provide preliminary data on their biological activities. Some aspects of this work have been summarized previously,[22] and further details of the biological work will be presented at a later date.

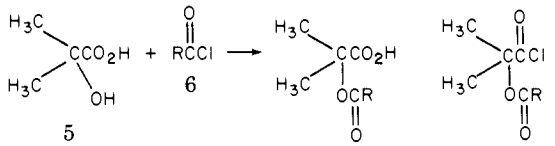
Chemical Synthesis. The key intermediates in the synthesis described in this paper are the homologous 2-acyloxyisobutyryl chlorides **8**. After exploring several possible routes for their routine synthesis, it was found that the direct heating of equimolar amounts of 2-hydroxyisobutyric acid **5** and the appropriate acyl chloride **6** at 50–80° with stirring for roughly 2 h without any added solvent led essentially quantitatively to the corresponding 2-acyloxyisobutyric acid **7**. This reaction was most conveniently monitored by infrared spectroscopy in carbon tetrachloride. Characteristically, the hydroxyl and acyl chloride bands (3600 and 1805 cm^{-1}) disappear and finally two closely spaced ester (1740–1745 cm^{-1}) and carboxyl (1720 cm^{-1}) bands remain, the latter usually being slightly more intense. In a number of cases (see Table I) the acyloxy acids **7** have been isolated in crystalline form for characterization, but for routine purposes this is not important and the crude product can be used directly in the next step.



Several long-chain 2-acyloxyisobutyric acids have been described in the patent literature via the pyridine-catalyzed condensation of **5** and **6**, and some of these compounds and their salts are claimed to be useful as preservatives in bakery products[23] and as antimicrobial agents.[24] 2-Stearoyloxypropionic acid has recently been prepared in high yield from lactic acid and stearoyl chloride at 120° without base or solvent.[25]

Conversion of the 2-acyloxyisobutyric acids **7** to the corresponding acid chlorides **8** is conveniently brought about through treatment with 2 equiv of thionyl chloride at 55–75° without added solvent. Once again, the reaction is easily monitored by infrared spectroscopy and is judged to be complete when the carboxyl band has been com-

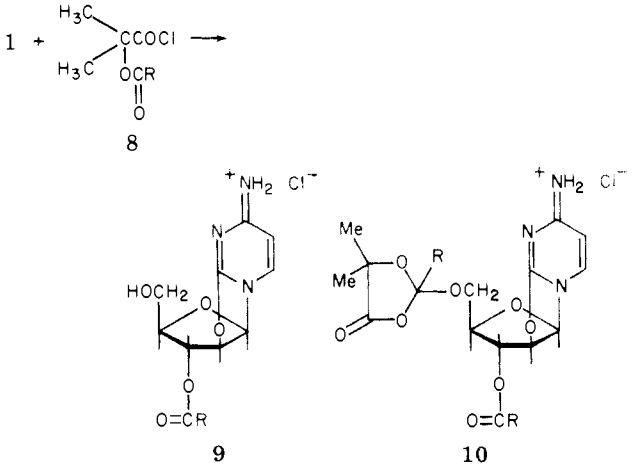
pletely replaced by an acyl chloride band at 1800–1805 cm^{-1}. Characteristically this band and the well-separated ester band at 1745 cm^{-1} are of roughly equal intensity. The lower homologues of 8 (C_4–C_8 acyl groups) can be readily isolated by distillation and small samples of the longer chain homologues (C_{10}–C_{14}) can be purified by short-path high-vacuum distillation; several of the still higher homologues are crystalline. If attempted distillation is prolonged, some decomposition appears to occur. Even the distilled products frequently give elemental analyses that are not totally satisfactory although their NMR spectra are entirely as expected and show the presence of both the *gem*-dimethyl group as a six-proton singlet at 1.54 ppm and the typical pattern of a fatty acid derivative. Once again, for routine purposes there is no need to purify the final product and it is sufficient to remove excess thionyl chloride and hydrogen chloride by stirring at room temperature at about 40 mm of pressure for several hours. In order to avoid excessive darkening of the reaction mixture it is necessary to use a good quality of thionyl chloride and to not allow the temperature to go above 80°. In the case of some of the unsaturated fatty acids, particularly dec-2-enoic acid, severe discoloration of the reaction mixture took place during preparation of the 2-acyloxyisobutyryl chloride. In these cases it is advisable to keep the temperature below 50° and, since further decomposition accompanied attempted distillation, the crude product, which gave a satisfactory infrared spectrum, was used directly in the next step. The physical properties of a representative number of the purified acids 7 and acid chlorides 8 are given in Table I.



5 6 7 8

After some experimentation it was found that the reaction between cytidine and the 2-acyloxyisobutyryl chlorides 8 could be conducted most economically using 2.5 molar equiv of 8 in acetonitrile (roughly 2 ml/mmol of cytidine). The reaction conditions are generally dependent upon the chain length of the acyloxy group of 8. Thus the short-chain (C_2–C_4) derivatives react quite rapidly at room temperature and once the cytidine has all dissolved giving a clear solution the reaction can be worked up. The intermediate chain derivatives (C_6–C_{14}) are advantageously allowed to react at 37–40° and usually lead to an initial clear, or nearly clear, solution followed by heavy precipitation of the crude product. The long-chain derivatives (C_{16}–C_{22}) of 8 are somewhat less reactive and require temperatures of 50–80° (see Table II). In these cases dissolution of the cytidine and precipitation of the product occur simultaneously, and while a change in the physical appearance of the mixture is apparent, completion of the reaction can only be judged by thin-layer chromatography of a representative aliquot containing both solid and liquid.

Our previous work has shown that the reaction of 1 with 2-acetoxyisobutyryl chloride (2a, 8, R = CH_3) leads under mild conditions to the anhydronucleoside 10 (R = Me) in which the 5'-hydroxyl group is converted to the 2,5,5-trimethyl-1,3-dioxolan-4-on-2-yl ether.[13d] This labile ether can be readily cleaved by mild acid to the corresponding 5'-hydroxy compound (9, R = Me). The formation of analogous 5'-*O*-dioxolanone ethers (10, R = Et, Pr, *i*-Pr) can also be shown to occur during reactions of cytidine with



8 9 10

the lower acyl derivatives of 8 (R = Et, Pr, *i*-Pr) and these substances can be isolated from the reaction mixtures by precipitation with ether. The presence of the dioxolanone function is apparent from the NMR spectra of the crude products, which characteristically show the *gem*-dimethyl group as two three-proton singlets near 1.4 ppm.[13d,20,21] The carbonyl group of the dioxolanone moiety is also apparent from its infrared absorption at 1800–1810 cm^{-1}. Since the dioxolanone group includes a chiral center, the derivatives 10 are diastereomeric mixtures and are not readily isolated in pure form. By brief treatment with dilute methanolic hydrogen chloride the dioxolanone grouping is rapidly cleaved giving the desired 5'-unsubstituted derivatives 9 which can then be purified by crystallization (procedure B). Dioxolanone formation does not appear to be a significant event using 2-acyloxyisobutyryl chlorides larger than the hexanoate.

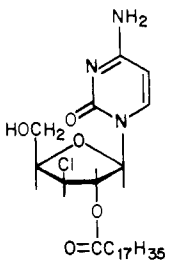
For reactions which do not lead to an intermediate 5'-dioxolanone, isolation of the crude product is completed by addition of ether, and the pure 2,2'-anhydro-1-(3'-*O*-acyl-β-D-arabinofuranosyl)cytosine hydrochloride (9, R = C_5–C_{22}) is obtained by direct crystallization (procedure A). In a number of cases the crude ether precipitates have been shown by NMR spectroscopy to be completely pure, but all the yields shown in Table II refer to recrystallized materials. In general the saturated acyl derivatives can be readily isolated in pure form in yields of 36–85% without any serious effort to rework mother liquors. In view of the high polarity of the products and their tendency to undergo partial decomposition in the presence of active adsorbants, chromatography on silicic acid, etc., has been avoided as a method of purification. The crude products that were derived from reactions that had to be run at higher temperatures (60–80°) usually contained minor, less polar by-products in addition to the desired products 9. Usually these by-products could be quite efficiently removed by crystallization from methanol, particularly in the presence of 10% of benzene, but in the case of the higher acyl derivatives (C_{20}–C_{22}) this was not entirely satisfactory. In those cases it was found that heating a suspension of the crude product in benzene for 30 min selectively removed much of the by-product. In the case of the stearoyl derivative (9, R = $C_{17}H_{35}$) the by-product was isolated in pure form by crystallization and was shown to be 1-(3-chloro-3-deoxy-2-*O*-stearoyl-β-D-xylofuranosyl)cytosine hydrochloride (11). The presence of the chloro function was apparent from elemental analysis and the typical ultraviolet spectrum of cytidine showed that the product was not an anhydronucleoside. The downfield position of $C_2'H$ (5.36 ppm in Me_2SO-d_6) relative to that in various 3'-*O*-acyl araC derivatives (4.07

ppm)[26] and the essentially identical chemical shifts of the $C_{5'}$ protons in both compounds show that the single stearoyl group is located at $C_{2'}$, and by analogy with our previous work[20,21] we assume the D-xylo configuration, which will arise by attack of chloride ion at $C_{3'}$ of the acetoxonium ion analogous to **3**.

The reactions of several unsaturated fatty acyl derivatives of 8 (undec-10-enoyl, oleoyl, elaidoyl, and erucoyl) were perfectly analogous to those of their saturated counterparts, although the isolated yields were slightly lower. The reaction using crude 2-(dec-2-enoyloxy)isobutyryl chloride (**8**, R = $CH{=}CHC_7H_{15}$), however, was very slow and was accompanied by extensive discoloration. In order to remove some tenacious impurities it was necessary to chromatograph the mother liquors on silicic acid and the yield of pure **9** (R = $CH{=}CHC_7H_{15}$) was only 17%. In a similar way the crude reaction mixture during synthesis of **9** (R = $CH_2C_6H_5$) looked quite clean by TLC but the pure, crystalline product could only be obtained in low yield. The reaction of cytidine with 2-benzoyloxyisobutyryl chloride (**8**, R = C_6H_5) was anomalous since the desired product (**9**, R = C_6H_5) could only be obtained in 15% yield from a reaction at 80° while 70% unreacted cytidine was recovered. By conducting the reaction at 37° for 26 days the starting material was consumed, but the major product was an anhydronucleoside that was considerably less polar than **9** (R = C_6H_5). Since this substance was not readily crystallized, the 2,2'-anhydro linkage was selectively cleaved by treatment with sodium bicarbonate–sodium carbonate in aqueous dioxane[26] giving 1-[3-*O*-benzoyl-5-*O*-(2-benzoyloxyisobutyryl)-β-D-arabinofuranosyl]cytosine (**12**) which was readily identified by NMR spectroscopy. The formation of 5'-*O*-(2-acetoxyisobutyrate) esters has previously been encountered during reactions with other nucleosides,[20,21] but this is the only example of such an ester that we have observed in the cytidine series. In spite of these occasional rather low yields when dealing with certain unsaturated or aromatic esters, the reactions described above make this a most versatile route to a wide range of 3'-*O*-acylated derivatives of cycloC and, as will be shown in an accompanying paper,[26] to the related 3'-*O*-acyl araC's.

All of the saturated and most of the unsaturated 3'-*O*-acyl cycloC's show the typical ultraviolet spectra shown by cycloC itself, with maxima close to 232 and 263 nm (see Table II). Only in the case of the α,β-unsaturated esters (benzoyl and dec-2-enoyl) was this pattern perturbed, the low-wavelength absorption then being of increased intensity and, in the latter case, shifted to 212 nm due to the contribution of the ester itself. With the exception of the characteristic patterns for the acyl fragments, the NMR spectra of all the 3'-*O*-acyl cycloC derivatives (in Me_2SO-d_6) were almost superimposable. Typically the $C_{5'}$ protons were magnetically nonequivalent and both $C_{1'}H$ and $C_{2'}H$ appeared as clean doublets since $J_{2',3'} = 0$. The spectra of several typical compounds are described more fully in the Experimental Section and a complete tabulation of the results can be made available to interested parties. It is interesting that, in spite of the ionic nature of the products, mass spectrometry is still a useful tool for confirmation of structure. Even the longer chain 3'-*O*-acyl derivatives show small but distinct molecular ions corresponding to the free base form of the anhydronucleoside, and more intense peaks corresponding to the loss of RCO and RCO_2 from that molecular ion are readily apparent. In the cases of the shorter acyl derivatives a careful examination of the spectra also reveals the presence of very small peaks corresponding to molecular ions of diacylated free base anhydronucleosides. This is so in highly purified samples and is apparently the consequence of intermolecular transesterification similar to what has previously been observed with 2,2'-anhydro-1-(3'-*O*-acetyl-β-D-arabinofuranosyl)uracil by Ogilvie et al.[29]

Finally, it has been demonstrated by Mikhailopulo et al.[27] that *O*-acetylsalicyloyl chloride (**13**) reacts with 1,2- and 1,3-diols to form acetoxyalkyl chlorides in much the same way as does **2a**. The reactions of **13** with alcohols have also been examined by Ruchardt and Rochlitz.[28] It was of interest to see whether this reagent could also be used for the conversion of cytidine to **4** or a 5' derivative thereof. The reaction of cytidine with commercially available **13** in acetonitrile gave a homogeneous reaction mixture in 2 h at 80°. While the reaction contained a number of by-products, several crystallizations from methanol–ethyl acetate gave pure **4** in a yield of 42%. This yield does not compare favorably with the 75% that can be obtained using **2a**, but the method is of interest since there is no indication for the formation of any 5' derivative. Since 2,2'-anhydro-(1-β-D-arabinofuranosyl)cytosine hydrochloride (cycloC) itself is easier to isolate than its 3'-*O*-acetyl derivative **4**, another reaction was investigated. In this case complete reaction had not occurred between cytidine and **13** at room temperature after 16 h but was brought to completion by subsequent brief heating at 70°. Following ether precipitation of crude **4** the acetyl group was hydrolyzed with methanolic hydrogen chloride and after two crystallizations pure cycloC hydrochloride was obtained in an overall yield of 65%. It thus appears that while **13** is considerably less reactive than **2a** (which reacts completely with cytidine at room temperature within 3 h), it does provide an alternate route to cycloC. In view of the lower reactivity and somewhat lower yields we have not investigated the higher acyl homologues of **13**.



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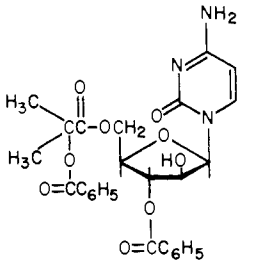

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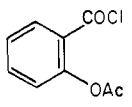

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Biological Studies. The various 3'-*O*-acyl derivatives of cycloC hydrochloride (**9**) prepared as above have been the subject of a fairly broad biological screen. In general this series has shown low or negligible activity against a representative spectrum of bacteria and fungi (minimum inhibitory concentrations generally greater than 100 μg/ml). They do, however, show significant cytotoxicity against HeLa cells in tissue culture, antiviral activity against DNA viruses (vaccinia and Herpes) but not against RNA viruses (polio, measles, and influenza Ao/WNS), and antitumor activity against L1210 leukemia in mice. The results of these studies are summarized in Table II, the following general protocols being used.

Cytotoxicity. Following the general procedure of Geran et al.[30] HeLa cells (10^5) were incubated in MEM supplemented with 10% bovine serum for 2 days at 37°. The medium was removed and fresh medium containing serial dilutions of the test compound in the same medium was added to the cell cultures. After 48 h the cells were dispersed with 0.02% trypsin and 0.04% versene and counted using a Model B Coulter counter. Values of ED_{50}

Table II. 3′-Acyl Derivatives of 2,2′-Anhydro-1-(β-D-arabinofuranosyl)cytosine Hydrochloride

Structure 9	Formula[a]	Mol wt	Mp, °C	Yield, %	Rxn conditions[b]	Purifcn method[c]	Uv (MeOH)$^{H^+}$, λ_{max} (ε)	Cytotoxicity,[e] ED_{50}, μg/ml	ED_{50}, μM[e] Vaccinia	ED_{50}, μM[e] Herpes	L1210 leukemia,[e] % ILS (30-day survivors) 200 mg/kg	500 mg/kg	1000 mg/kg
(a) Saturated													
Unsubstituted[i]	$C_9H_{12}N_3O_4Cl$	261.68	262–264	73[e]	RT, 3 h	B, MeOH-Me_2CO	231 (9900), 263 (10800)	0.05	0.41	0.70	25 (0/8)	67 (0/8)	89 (0/8)
Acetyl[i]	$C_{11}H_{14}N_3O_5Cl$	303.70	254–255	75	RT, 3 h	B, MeOH-Me_2CO	232 (9500), 263 (11000)	0.18	0.73	2.25	37 (0/8)		87 (0/8)
Propionyl	$C_{12}H_{16}N_3O_5Cl$	317.73	258–259	54	80°, 15 min	B, EtOH	230 (9300), 262 (10300)	0.1	0.60	2.55	29 (0/8)		
Butyryl[i]	$C_{13}H_{18}N_3O_5Cl$	331.75	242–244	59	RT, 2.5 h	B, MeOH	230 (9300), 264 (10800)	0.12	0.59	2.05	30 (0/8)		
Isobutyryl	$C_{13}H_{18}N_3O_5Cl$	331.75	253–255	85	RT, 2.5 h	B, MeCN	230 (9600), 263 (10900)	0.20	1.55	8.8	22 (0/8)		
Hexanoyl	$C_{15}H_{22}N_3O_5Cl$	359.81	232–235	72	37°, 3.5 h	A, MeOH	230 (9600), 263 (11500)	0.10	0.23	1.55	40 (0/8)		
Octanoyl	$C_{17}H_{26}N_3O_5Cl$	387.86	236–239	68	37°, 2.5 h	A, MeOH	231 (9700), 263 (10800)	0.10	0.19	0.72	34 (0/7)	−93 (0/8)	−100 (0/8)
Nonanoyl	$C_{18}H_{28}N_3O_5Cl$	401.91	237–238	36	37°, 2 h	A, MeOH	232 (10600), 263 (11400)	0.05	0.29	0.40	33 (0/8)		
Decanoyl	$C_{19}H_{30}N_3O_5Cl$	415.93	227–229	53	37°, 16 h	A, MeOH	232 (9400), 263 (10800)	0.20	0.15	1.05	35 (0/8)		
Undecanoyl	$C_{20}H_{32}N_3O_5Cl$	429.96	229–230	65[g]	45°, 2 h	A, MeOH	232 (10000), 264 (11200)	0.06	0.19	0.46	87 (0/8)	>237 (4/8)	
Dodecanoyl	$C_{21}H_{34}N_3O_5Cl$	443.98	232–233	52	37°, 16 h	A, MeOH	232 (9500), 263 (10800)	0.10	0.64	0.64	40 (0/8)		
Myristoyl	$C_{23}H_{38}N_3O_5Cl$	472.03	235–237	68	37°, 16 h	A, MeOH	232 (9500), 264 (10400)	0.20	0.34	1.35	100 (0/8)		>280 (8/8)
Palmitoyl	$C_{25}H_{42}N_3O_5Cl$	500.10	219–221	39	80°, 16 h	A, MeOH	233 (10000), 264 (10900)	0.20	1.20	5.50	>191 (3/8)		−20 (0/8)
Stearoyl	$C_{27}H_{46}N_3O_5Cl$	528.15	218–220	79	60°, 7 h	A, MeOH	232 (9900), 264 (10700)	0.22	1.60	5.45	115 (0/8)		>257 (6/6)
Arachidyl	$C_{29}H_{50}N_3O_5Cl$	556.20	225–228	66	50°, 5 h	A, MeOH	232 (9800), 265 (10600)	0.22	>30	>30	>281 (5/7)	>202 (6/8)	>266 (8/8)
Behenoyl	$C_{31}H_{54}N_3O_5Cl$	584.25	220–221	59	60°, 5 h	A,[h] MeOH	232 (8400), 263 (9100)	0.38	18.50	>30	>169 (3/10)		>240 (8/3)
(b) Unsaturated													
Benzoyl	$C_{16}H_{16}N_3O_5Cl$	365.77	273–275	15	80°, 1 h	B, MeOH	233 (23700), 263 (12100)	0.20	5.90	8.20	17 (0/8)		
Phenylacetyl	$C_{17}H_{18}N_3O_5Cl$	379.82	231–233	14	37°, 16 h	A, MeOH	232 (10900), 264 (11300)	0.07	0.56	0.56	33 (0/8)		

Dec-2-enoyl	$C_{19}H_{28}N_3O_5Cl$	419.92	226–227	17	80°, 16 h	A, C, MeOH	212 (20600), 263 (11100)	0.06	0.15	0.21	−83 (toxic)
Undec-10-enoyl	$C_{20}H_{30}N_3O_5Cl$	427.94	231–232	34	37°, 16 h	A, MeOH	232 (9900), 264 (10800)	0.05	0.51	1.20	38 (0/8)
Oleoyl	$C_{27}H_{44}N_3O_5Cl$	526.13	231–232	32	55°, 16 h	A, EtOH	233 (10300), 264 (11200)	0.08	0.67	1.20	72 (0/8)
Elaidoyl	$C_{27}H_{44}N_3O_5Cl$	526.13	219–221	50	55°, 6 h	A, MeOH	233 (10200), 264 (11000)	0.09	0.90	1.90	>162 (2/8)
Erucoyl	$C_{31}H_{52}N_3O_5Cl$	582.24	225–226	46	55°, 5 h	A, MeOH	234 (10700), 265 (11600)	0.18	30	>30	>269 (7/8)

[a] All compounds analyzed correctly for C, H, and N. [b] In each case the reaction used 2.5–3.0 molar equiv of the 2-acyloxyisobutyryl chloride (8), generally in crude form (see text), per equivalent of cytidine in acetonitrile (2 ml/mmol of cytidine). [c] A, ether precipitation and crystallization from the indicated solvent; B, ether precipitation followed by treatment with 0.05 M methanolic HCl for 1–1.5 h, evaporation, ether precipitation, and crystallization from the indicated solvent. C, chromatography of mother liquors on a column of silicic acid using 10–20% methanol in chloroform followed by crystallization. [d] Against BGM cells in tissue culture. See text. [e] See text for methodology. [f] Including acidic hydrolysis of the 3'-*O*-acetyl group.[13d] [g] 80% crude product completely pure by NMR. [h] Crude ether precipitate refluxed with benzene for 10 min to remove nonpolar impurities. [i] See ref 13d.

were determined graphically and are reported in Table II.

Antiviral. Preliminary screening was done using a pulp disk diffusion assay[31] with the BGM[32] line of African green monkey kidney cells infected with 200 plaque forming units (pfu) of the IHD strain of vaccinia virus, the HF strain of Herpes simplex virus, Type I Mahoney strain polio virus or measles virus, and with $LLCMK_2$ cells infected with influenza virus Ao/WSN in the presence of trypsin. The filter paper disk was impregnated with 50 μg of the test compound and antiviral activity was measured in terms of the diameter of the zone of plaque inhibition on day 5. Compounds that were positive in the above test were further studied by a quantitative plaque reduction assay. In this assay a confluent monolayer of BGM cells is infected with 100–200 pfu and then overlayed with agar medium [1.25% agar (Bact-agar, Difco) in minimal essential medium with 2.0% bovine serum] containing different concentrations of the compound to be tested. On day 4, the second overlay was added with the same medium plus neutral red (1:20000). The flasks were incubated; plaques were counted on day 6. The 50% reduction in concentration of the compound (ED_{50}) was determined by plotting percentage inhibition vs. compound concentration on probability paper. The results against Herpes and vaccinia viruses are reported in Table II in terms of the micromolar concentration of test compound necessary for 50% reduction of viral plaque forming units.

Anti-L1210 Leukemia. BDF_1 mice were infected by intraperitoneal injection with $1–2 \times 10^5$ L1210 leukemic cells. After 24 h a single dose of the test substance as a solution or suspension in an aqueous medium containing 0.9% sodium chloride, 0.4% polysorbate 80, 0.5% low-viscosity sodium carboxymethylcellulose, and 0.9% benzyl alcohol was administered by intraperitoneal (ip) injection and the mice were housed with normal diet for a total of 30 days. The average life span of a control group of infected mice was close to 8 days and the effectiveness of the drug was expressed in terms of the percent increase in life span (% ILS) of the treated group (eight to ten animals) relative to that of the controls. The results from a standard dose of 200 mg/kg are reported in Table II, and in certain cases higher doses were also examined. The numbers in parentheses following the % ILS figures refer to the number of 30-day survivors within the test group. Clearly, whenever animals were still alive at the termination of the experiment the indicated % ILS is a minimum figure and is represented (e.g., for 3'-palmitoyl cycloC) as >191% (3/8). A negative % ILS figure indicates a shortening of average life span and is indicative of drug toxicity.

From Table II it can be seen that all the 3'-*O*-acyl derivatives of cycloC·HCl were of roughly comparable cytotoxicities toward HeLa cells, the values of ED_{50} varying between 0.05 and 0.38 μg/ml. With respect to antiviral activity, vaccinia virus was always somewhat more sensitive toward the test compounds than was Herpes virus. The addition of 3'-*O*-acyl functions (C_2–C_4) led to a modest decrease in antiviral activity relative to that of cycloC itself. Further increases in the length of the acyl chain, however, were accompanied by enhanced antiviral activity with the saturated C_6–C_{12} esters being roughly twice as effective as cycloC on a molar basis. Still further increases in chain length led to reduced activity, the C_{20} and C_{22} esters showing no significant antiviral effect. This reduced activity of the higher esters is almost certainly related to the very low solubilities of these substances in aqueous media. Even at the low concentrations examined, dilution of methanol solutions of the higher esters with culture medium led to visible precipitation of the test compounds.

To confirm this idea, solubilized preparations of a number of compounds were examined.[33] Such preparations of the shorter chain length compounds (C_8–C_{14}) showed ED_{50} values very similar to, or slightly higher than, those of the free compounds. A solubilized preparation of 3'-*O*-behenoyl cycloC (C_{22}), however, now showed an ED_{50} toward vaccinia virus of 2.80 μM, an increase in activity of 6.6-fold. This strongly supports the concept that lack of solubility was a limiting factor with the very long chain esters. Long-chain unsaturated esters (oleoyl, elaidoyl) appear to be somewhat more active than their saturated C_{18} counterpart (stearoyl).

The activities of the various compounds against L1210 leukemia in mice are also summarized in Table II, which also provides a comparison with the effects of cycloC (unsubstituted) itself. While cycloC is a compound of considerable interest, it may be noted that when administered as a single ip dose 24 h following L1210 inoculation significant protection is only provided by large doses. As an extension of Table II, the observed % ILS of cycloC at doses of 2000 mg/kg was >151 (3/8) while toxicity was apparent at 3000 mg/kg. The effectiveness of cycloC can, however, be substantially increased by the use of different dosage regimens.[15b] From Table II it can be seen that in general the lower 3'-*O*-acyl homologues (C_2–C_{10}) of cycloC do not show any marked advantage over the parent compound as judged from the results following single ip injections. In certain cases (e.g., the 3'-octanoyl ester) marked toxicity was observed once the dose was increased beyond the standard 200 mg/kg. The longer chain saturated esters, however, showed greatly enhanced activity with frequent 30-day survivors. In most cases there was little sign of toxicity since excellent responses were obtained at 1000 mg/kg or even higher. The apparent toxicity of the 3'-*O*-palmitoyl ester at high doses seem anomalous and is not readily explained. The unsaturated esters followed a roughly similar pattern, the shorter chain derivatives having modest activity and the higher homologues such as the 3'-*O*-elaidoyl (trans-Δ^9-C_{18}) and 3-*O*-erucoyl (cis-Δ^{13}-C_{22}) esters being highly active. The considerably greater activity of the elaidoyl ester relative to its cis counterpart (oleoyl) and the striking toxicity shown by the 3'-*O*-dec-2-enoyl derivative are interesting and deserve further study.

At this point it is not clear whether the increased activity shown by the higher esters is a consequence of an increase in cellular penetration, a change in tissue distribution, or some other factor. Certainly some of the esters show very prolonged activity since subcutaneous administration of, e.g., 3'-*O*-myristoyl cycloC (**9**, R = $C_{13}H_{27}$) 10 days prior to L1210 cell infection still affords a 64% ILS at a dose of 500 mg/kg. It seems not unlikely that the very long chain esters, due to their low solubilities, are partially precipitating at the site of injection and are then only slowly dissolved, leading to a continuous slow release of the drug. More detailed studies on the use of different modes of administration, different dosage regimens, and different tumor and virus systems will be presented at a later date.

Experimental Section

General Methods. Thin-layer chromatography (TLC) was conducted on 5 × 20 cm glass plates coated with a 250-μ layer of silica gel HF from Analtech Corp. and preparative TLC on 20 × 100 cm glass plates coated with a 1.3-mm layer of Merck silica gel GF. Column chromatography was done using Merck silica gel with 0.05–0.20 mm particles. Nuclear magnetic resonance (NMR) spectroscopy was done using a Varian HA-100 instrument and spectra are recorded in parts per million downfield from an internal standard of tetramethylsilane. Mass spectra were obtained using an Atlas CH-4 instrument fitted with a direct inlet system. Elemental and other instrumental analyses were obtained by the staff of the Analytical Laboratory of Syntex Research or from Dr. A. Bernhardt, Elbach über Engelskirchen, Germany. Melting points were obtained using a hot-stage microscope and are corrected. We express our thanks to Drs. M. L. Maddox and L. Tökes and to Mrs. J. Nelson for their help with NMR and mass spectrometry.

Preparation of 2-Acyloxyisobutyric Acids (7). (a) **2-Myristoyloxyisobutyric Acid (7, R = $C_{13}H_{27}$).** A mixture of 2-hydroxyisobutyric acid (88.9 g, 0.86 mol) and myristoyl chloride (212.5 g, 0.86 mol) was stirred with gentle heating until a vigorous evolution of hydrogen chloride started, and heating was then stopped until gas evolution moderated. The solution was then reheated to 80° until gas evolution ceased (usually about 2-h total reaction time). The mixture was then stirred at a pressure of roughly 40 mm of mercury until no further gas evolution was observed. Upon cooling to room temperature overnight the entire mixture had crystallized giving 268.5 g (98%) of essentially pure **7** (R = $C_{13}H_{27}$) with mp 54–55°. Recrystallization of an analytical sample from hexane raised the melting point to 55–56°: NMR ($CDCl_3$) 0.86 (t, 3, terminal CH_3), 1.26 (s, 20, CH_2's), 1.56 (s, 6, CMe_2), 1.6 (m, 2, $COCH_2CH_2$), 2.28 (t, 2, J = 7 Hz, $COCH_2$), 10.05 ppm (br s, 1, COOH). See Table I for other data.

(b) **2-Behenoyloxyisobutyric Acid (7, R = $C_{21}H_{43}$).** Behenoyl chloride (287.2 g, 0.8 mol)[34] and 2-hydroxyisobutyric acid (83.2 g, 0.8 mol) were heated at 60–65° until gas evolution ceased. The solution was then maintained at 1 mm pressure for 30 min and cooled. Crystallization of the residue from hexane (500 ml) gave 205 g of pure product. The mother liquors were treated with charcoal, filtered, and concentrated giving a further 84.18 g (total yield 289.2 g, 85%) of **7** (R = $C_{21}H_{43}$) with mp 72–73°: NMR ($CDCl_3$) 0.86 (t, 3, CH_3), 1.23 (s, 36, CH_2's), 1.56 (s, 6, CMe_2), 1.6 (m, 2, $COCH_2CH_2$), 2.27 (t, 2, $COCH_2$), 10.2 ppm (br s, 1, COOH); mass spectrum (70 eV) m/e 426 (M^+), 340 (behenic acid), 323. See Table I for other data.

2-Acyloxyisobutyryl Chlorides (8). (a) **2-Decanoylisobutyryl Chloride (7, R = C_9H_{19}).** A mixture of decanoyl chloride (47.7 g, 0.25 mol) and 2-hydroxyisobutyric acid (26.8 g, 0.25 mol) was stirred and gradually heated to 80° at such a rate as to control gas evolution. When gas evolution was complete (2 h) the infrared spectrum showed only a clearly resolved double peak at 1720 and 1740 cm^{-1} in the carbonyl region. Without isolation of the crystalline acid (**7**, R = C_9H_{19}, mp 30.5–31°), thionyl chloride (36.5 ml, 0.50 mol) was added and, after the initial vigorous reaction had subsided, the mixture was heated at 75° until gas evolution was complete (2 h). Excess thionyl chloride was removed by heating at 50° and 40 mm Hg and then at 1 mm pressure. The residue (72.7 g) showed ir bands of equal intensity at 1745 and 1805 cm^{-1} and the crude light-colored product was suitable for direct use. For analytical purposes a 5-g sample was distilled twice [bp 140° (0.1 mm)] giving 3.37 g (66% overall) of pure product with an infrared spectrum essentially identical with that of the crude material: NMR ($CDCl_3$) 0.86 (t, 3, CH_3), 1.26 (s, 12, CH_2's), 1.60 (s, 6, CMe_2), 1.6 (m, 2, $COCH_2CH_2$), 2.35 ppm (t, 2, $COCH_2$). See Table I.

(b) **2-Stearoyloxyisobutyryl Chloride (8, R = $C_{17}H_{35}$).** A mixture of stearoyl chloride (75.73 g, 0.25 mol) and 2-hydroxyisobutyric acid (26.0 g, 0.25 mol) was stirred and gradually heated to 80° for 1.5 h, at which time the infrared spectrum showed carbonyl bands only at 1715 and 1740 cm^{-1}. In a separate experiment a portion of the mixture was evacuated at 40 mm pressure for 2 h and cooled leaving a crystalline residue. Recrystallization from hexane gave pure **7** (R = $C_{17}H_{35}$) with mp 60–61° (reported[23a] mp 59°). Thionyl chloride (73 ml, 1 mol) was added slowly to the crude acid through the condenser under anhydrous conditions while the mixture was maintained at 80° for 4 h. Excess thionyl chloride was removed by stirring overnight at 50° and 30 mm pressure and upon cooling to room temperature the entire residue crystallized giving 103 g of crude **8** (R = $C_{17}H_{35}$) that was suitable for direct use. Distillation was not successful but recrystallization from hexane gave pure **8** (R = $C_{17}H_{35}$) with mp 53–54°: NMR (CCl_4) 0.87 (t, 3, CH_3), 1.25 (s, 28, CH_2's), 1.60 (s, 6, CMe_2), 1.7 (m, 2, $COCH_2CH_2$), 2.28 ppm (t, 2, $COCH_2$); mass spectrum (70 eV) m/e 388, 390 (M^+), 352 (M^+ – HCl). See Table I.

(c) **2-Undec-10-enoyloxyisobutyryl Chloride [8, R = $(CH_2)_8CH{=}CH_2$].** A mixture of undec-10-enoyl chloride (66 g, 0.325 mol) and 2-hydroxyisobutyric acid (37.1 g, 0.36 mol) was heated at 50° until gas evolution ceased and the infrared spectrum (CCl_4) showed carbonyl bands at 1720 and 1740 cm^{-1}. Thionyl chloride (47.5 ml, 0.65 mol) was then added and the mixture was heated at 50° for 2 h. Excess thionyl chloride was removed at 50° and 40 mm of pressure overnight leaving an essentially quantitative yield of crude product as a light-colored syrup which showed carbonyl bands (CCl_4) of equal intensity at 1745 and 1810 cm^{-1}. The material could not readily be distilled and was used as such.

Reactions of 2-Acyloxyisobutyryl Chlorides with Cytidine. (a) **2,2'-Anhydro-1-(3'-*O*-undecanoyl-β-D-arabinofuranosyl)cytosine Hydrochloride (9, R = $C_{10}H_{21}$). Procedure A.** 2-Undecanoyloxyisobutyryl chloride (156 g, 0.536 mol) was added to a stirred suspension of cytidine (52.1 g, 0.214 mol) in anhydrous acetonitrile (430 ml). The mixture was stirred at 45° and after 40 min most of the cytidine had dissolved. Within 60 min, however, heavy precipitation of a white solid commenced and the mixture was kept at 45°. After a total of 2 h an aliquot (solid and liquid) was removed and triturated with ether. Examination of the precipitate by TLC using acetonitrile–0.1 M ammonium chloride (9:1) or 1-butanol–acetic acid–water (5:2:3) showed that no cytidine remained and an essentially single, less polar product was present. The reaction mixture was added with stirring to anhydrous ether (1 l.) and the resulting fine precipitate was collected by centrifugation.[35] The precipitate was washed several times with fresh ether and then dried in vacuo leaving 73.31 g (80%) of **9** (R = $C_{10}H_{21}$) that was completely pure by NMR spectroscopy and TLC. Crystallization from hot methanol gave 59.96 g (65%) of analytically pure product with mp 229–230°. NMR (Me_2SO-d_6)[36] 0.88 (t, 3, CH_3), 1.27 (s, 14, CH_2's), 1.60 (m, 2, $COCH_2CH_2$), 2.40 (t, 2, $COCH_2$), 3.34 (dd, 1, J_{gem} = 13 Hz, $J_{4',5'a}$ = 2 Hz, $C_{5'}aH$), 3.51 (dd, 1, $J_{4',5'b}$ = 2 Hz, $C_{5'}bH$), 4.45 (br s, 1, $C_4'H$), 5.41 (s, 1, $J_{2',3'}$ = 0 Hz, $J_{3',4'}$ < 1 Hz, $C_3'H$), 5.64 (d, 1, $J_{1',2'}$ = 6 Hz, $C_2'H$), 6.62 (d, 1, $C_1'H$), 6.70 (d, 1, $J_{5,6}$ = 7.5 Hz, C_5H), 8.31 (d, 1, C_6H), 9.20 and 9.66 ppm (br s, 1, NH_2); mass spectrum (70 eV) *m/e* 394 (M^+, free base + H), 393 (M^+, free base), 280 ($M^+ - C_8H_{17}$), 267 ($M^+ - C_9H_{19}$ + H), 224 (M^+ – RCO), 208 (M^+ – RCO_2), 561 (diester), 448 (diester – C_9H_{19} + H).

(b) 2,2'-Anhydro-1-(3'-*O*-isobutyryl-β-D-arabinofuranosyl)cytosine Hydrochloride (9, R = *i*-Pr). Procedure B. A mixture of cytidine (486 mg, 2 mmol) and 2-isobutyryloxyisobutyryl chloride (1.3 ml, 8 mmol) in acetonitrile (5 ml) was stirred at room temperature for 2.5 h and then heated to 70° for 10 min. During this time the mixture became homogeneous and then a small amount of crystalline product separated. Most of the solvent was evaporated in vacuo and the residue was triturated with ether giving 0.9 g of a precipitate that was shown by NMR to contain a 2-isopropyl-5,5-dimethyl-1,3-dioxolan-4-on-2-yl group (1.37 and 1.42, s, 3, CMe_2). This material was dissolved in 0.18 M methanolic hydrogen chloride (20 ml) and kept at room temperature for 40 min. The solvent was evaporated and the residue triturated with ether giving a white solid that was thoroughly washed with fresh ether and dried in vacuo. Crystallization from acetonitrile gave 560 mg (85%) of **9** (R = *i*-Pr) with mp 253–255°: NMR (Me_2SO-d_6) 1.12 (d, 6, *J* = 7 Hz, $CHMe_2$), 2.59 (m, 1, $CHMe_2$), 3.31 (dd, 1, J_{gem} = 12 Hz, $J_{4',5'a}$ = 2.5 Hz, $C_{5'}aH$), 3.54 (dd, 1, $J_{4',5'b}$ = 2 Hz, $C_{5'}bH$), 4.39 (br s, 1, $C_4'H$), 5.38 (s, 1, $J_{2',3'}$ = 0 Hz, $J_{3',4'}$ < 1 Hz, $C_3'H$), 5.61 (d, 1, $J_{1',2'}$ = 6 Hz, $C_2'H$), 6.61 (d, 1, $C_1'H$), 6.69 (d, 1, $J_{5,6}$ = 7 Hz, C_5H), 8.26 (d, 1, C_6H), 9.25 and 9.79 ppm (br s, 1, NH_2). See Table II for other data.

(c) 2,2'-Anhydro-1-(3'-*O*-stearoyl-β-D-arabinofuranosyl)cytosine Hydrochloride (9, R = $C_{17}H_{35}$). A mixture of cytidine (50 g, 0.205 mol) and crude 2-stearoyloxyisobutyryl chloride (from 0.52 mol of 2-stearoyloxyisobutyric acid and 1 mol of thionyl chloride at 60° for 2.5 h followed by removal of excess thionyl chloride at 60° and 30 mm of pressure) in acetonitrile (1 l.) was stirred at 60°. A clear reaction mixture was never observed but TLC (1-butanol–acetic acid–water), 5:2:3) showed disappearance of cytidine after 6 h. After 7 h the mixture was cooled and the precipitate was collected by filtration and washed well with ether. After drying in vacuo the yield of **9** (R = $C_{17}H_{35}$), which was contaminated with a minor, less polar product, was 120.7 g. Crystallization of this material from methanol with addition of 10% benzene before cooling gave 86.0 g (79%) of pure **9** (R = $C_{17}H_{35}$) in two crops with mp 218–220°: NMR (Me_2SO-d_6) 0.84 (t, 3, CH_3), 1.25 (s, 28, CH_2's), 1.5 (m, 2, $COCH_2CH_2$), 2.37 (t, 2, $COCH_2$), 3.30 (dd, 1, J_{gem} = 12 Hz, $J_{4',5'a}$ = 3 Hz, $C_{5'}aH$), 3.52 (dd, 1, $J_{4',5'b}$ = 2.5 Hz, $C_{5'}bH$), 4.41 (br s, 1, $C_4'H$), 5.39 (br s, 1, $J_{2',3'}$ = 0 Hz, $J_{3',4'}$ < 1 Hz, $C_3'H$), 5.61 (d, 1, $J_{1',2'}$ = 6 Hz, $C_2'H$), 6.58 (d, 1, $C_1'H$), 6.58 (d, 1, $J_{5,6}$ = 7 Hz, C_5H), 8.25 (d, 1, C_6H), 9.21 and 9.47 ppm (br s, 1, NH_2); mass spectrum (70 eV) *m/e* 491 (M^+, free base), 462, 448, 407, 392 (fragmentation of stearoyl group), 284 (stearic acid), 267 ($C_{17}H_{35}CO$), 208 (M – stearic acid). See Table II for other data.

The final mother liquors were evaporated and the residue was triturated with ether. Further crystallization of the residue from methanol–benzene (9:1) gave 5.65 g of the almost pure, less polar by-product mentioned above. Recrystallization from methanol then gave 3.70 g (3%) of pure 1-(3-chloro-3-deoxy-2-*O*-stearoyl-β-D-xylofuranosyl)cytosine hydrochloride (**11**) with mp 177–179°: λ_{max} (MeOH, H^+) 212 nm (ε 9000), 282 (13400); NMR (Me_2SO-d_6) 0.85 (t, 3, CH_3), 1.25 (s, 28, CH_2's), 1.55 (m, 2, $COCH_2CH_2$), 2.38 (t, 2, $COCH_2$), 3.78 (d, 2, $J_{4',5'}$ = 5 Hz, $C_{5'}H_2$), 4.44 (dt, 1, $J_{3',4'}$ = $J_{4',5'}$ = 4 Hz, $C_4'H$), 4.74 (dd, 1, $J_{2',3'}$ = 2.5 Hz, $C_3'H$), 5.36 (dd, 1, $J_{1',2'}$ = 2.5 Hz, $C_2'H$), 5.82 (d, 1, $C_1'H$), 6.25 (d, 1, $J_{5,6}$ = 7.5 Hz, C_5H), 8.08 (d, 1, C_6H), 8.84 and 9.94 ppm (br s, 1, NH_2); mass spectrum (70 eV) *m/e* 527, 529 (M^+), 492 (M – HCl), 416, 418 (M – base). Anal. C, H, N, Cl.

Reaction of Cytidine with 2-Benzoyloxyisobutyryl Chloride. (a) A mixture of cytidine (486 mg, 2 mmol) and 2-benzoyloxyisobutyryl chloride (1.8 g, 8 mmol, distilled) in acetonitrile was heated at 80° for 1 h and then unreacted cytidine (0.33 g) was removed by filtration. The filtrate was evaporated and the residue triturated with ether giving a dry solid that was treated with 0.18 M methanolic hydrogen chloride (10 ml) at room temperature for 40 min. After evaporation to dryness, the residue was washed with ether and crystallized from methanol giving 110 mg (15%, 45% based on unrecovered cytidine) of **9** (R = C_6H_5) with mp 275° dec: NMR (Me_2SO-d_6) 3.36 (dd, 1, J_{gem} = 12 Hz, $J_{4',5'a}$ = 2.5 Hz, $C_{5'}aH$), 3.62 (dd, 1, $J_{4,5'b}$ = 2 Hz, $C_{5'}bH$), 4.64 (m, 1, $C_4'H$), 5.61 (s, 1, $J_{2',3'}$ = 0 Hz, $J_{3',4'}$ < 1 Hz, $C_3'H$), 5.84 (d, 1, $J_{1',2'}$ = 6 Hz, $C_2'H$), 6.65 (d, 1, $C_1'H$), 6.69 (d, 1, $J_{5,6}$ = 7 Hz, C_5H), 7.6 (m, 3, Ar), 8.01 (dd, 2, Ar), 8.30 (d, 1, C_6H), 9.26 and 9.77 ppm (br s, 1, NH_2).

(b) A mixture of cytidine (486 mg, 2 mmol) and 2-benzoyloxyisobutyryl chloride (1.8 g, 8 mmol) was stirred at 37° for 26 days, a clear solution resulting after 21 days. Methanol (5 ml) was added and after 40 min at room temperature the solvents were largely removed and the residue was triturated with ether giving 655 mg of a crude, dry precipitate. This material was dissolved in a mixture of water (30 ml) and dioxane (21 ml) containing sodium bicarbonate (600 mg) and sodium carbonate (450 mg) and stored at room temperature for 140 min. After evaporation of the solvent the residue was extracted with methanol and the extracts were purified by preparative TLC using chloroform–methanol (6:1) giving 278 mg (26%) of 1-[3-*O*-benzoyl-5-*O*-(2-benzoyloxyisobutyryl)-β-D-arabinofuranosyl]cytosine (**12**) as a TLC homogeneous foam: λ_{max} (EtOH) 231 nm (ε 34000), 274 (11400); NMR ($CDCl_3$) 1.70 (s, 6, CMe_2), 4.40 (m, 2, $C_{5'}H_2$), 4.70 (m, 1, $C_4'H$), 4.99 (d, 1, $J_{1',2'}$ = 3 Hz, $C_2'H$), 5.22 (br s, 1, $C_3'H$), 5.72 (d, 1, $J_{5,6}$ = 7 Hz, C_5H), 6.05 (d, 1, $C_1'H$), 7.38 (m, 6, Ar), 7.65 (d, 1, C_6H), 7.93 ppm (dd, 4, Ar). Anal. H, N; C: calcd, 60.33; found, 59.81.

Reaction of Cytidine with *O*-Acetylsalicyloyl Chloride (13). (a) A mixture of cytidine (4.86 g, 20 mmol) and **13** (15.9 g, 80 mmol) in acetonitrile (200 ml) was stirred at 80° for 2 h giving a homogeneous solution. The solvent was then largely removed in vacuo and the residue was triturated with several portions of ether (200 ml each) giving a granular solid. Two crystallizations of this material from methanol–ethyl acetate gave 2.52 g (42%) of 2,2'-anhydro-1-(3'-*O*-acetyl-β-D-arabinofuranosyl)cytosine hydrochloride (**9**, R = CH_3) with mp 252–254° that was chromatographically and spectroscopically identical with an authentic sample.

(b) A mixture of cytidine (24.3 g, 100 mmol) and *O*-acetylsalicyloyl chloride (49.65 g, 250 mmol) in acetonitrile (200 ml) was stirred overnight at room temperature and then heated to 70° for 30 min giving a homogeneous solution. The solvent was

largely evaporated and the residue was stirred with ether (500 ml) for 2.5 h giving a granular solid that was washed with ether and dried in vacuo. This material (46 g) was dissolved in 0.3 M methanolic hydrogen chloride (750 ml) and stirred for 3 days at room temperature, during which time a crystalline product separated. Recrystallization from methanol gave 16.97 g (65%) of pure 2,2'-anhydro-1-(β-D-arabinofuranosyl)cytosine hydrochloride with mp 262–264° that was in all ways identical with an authentic sample.

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(35) Certain of the crude products at this stage require collection by centrifugation, while others are readily filtered.
(36) The NMR spectra for the nucleoside moieties of all the 3'-*O*-acyl cyclocytidine derivatives are essentially identical. A full tabulation is available to anyone interested in further details.