and the residue was triturated with ether (500 ml) giving a fine precipitate that was collected by centrifugation, washed with ether, and dried in vacuo (26 g). The somewhat colored residue was dissolved in hot methanol, treated with charcoal (3 g), filtered, concentrated, and allowed to crystallize giving 19.11 g of pure product. The mother liquors were evaporated to dryness and applied in chloroform to a column containing 250 g of silicic acid. Elution of the column with chloroform containing 5 and 10% methanol in chloroform gave a further 10.7 g of crude material that was recrystallized from methanol giving 6.49 g of pure product (total yield 25.6 g, 57%) with mp 155–156°: NMR ($CDCl_3$) 0.8–2.45 (m, 78, CH_2's and CH_3's), 3.90 (dd, 1, J_{gem} = 12 Hz, $J_{4',5'a}$ = 5 Hz, $C_{5'a}H$), 4.24 (dd, 1, $J_{4',5'b}$ = 4 Hz, $C_{5'b}H$), 4.47 (m, 1, C_4·H), 5.30 (t, 4, J = 5 Hz, $CH_2CH{=}C$), 5.35 (br s, 1, $C_{3'}$H), 5.83 (d, 1, $J_{1',2'}$ = 6 Hz, $C_{2'}$H), 6.93 (d, 1, $C_{1'}$ H), 7.12 (d, 1, $J_{5,6}$ = 7 Hz, C_5H), 8.15 (d, 1, C_6H), 8.45 and 9.95 ppm (br s, 1, NH_2); mass spectrum (70 eV) m/e 865 (M^+, free base), 545 (M – RCO), 527 (m/e 545 – H_2O), 322 (RCO). Anal. C, H, N. See Table I for other data.

2,2'-Anhydro-1-[3'-*O*-acetyl-5'-*O*-(4-methylbicyclo[2.2.2]-oct-2-ene-1-carbonyl)-β-D-arabinofuranosyl]cytosine Hydrochloride (3c). 4-Methylbicyclo[2.2.2]oct-2-ene-1-carbonyl chloride (**2c**, from 700 mg of the acid, 4.2 mmol)[14] and **1** (250 mg, 0.82 mmol) were stirred together in dimethylacetamide (15 ml) at room temperature for 7 days giving a clear solution. Most of the solvent was removed under high vacuum and the residue was triturated with ether giving a crystalline residue. Recrystallization from chloroform gave 308 mg (83%) of **3c** with mp 231–235° dec: NMR (Me_2SO-d_6) 1.12 (s, 3, CH_3), 1.2–1.9 (m, 8, CH_2's), 2.11 (s, 3, OAc), 4.09 (dd, 1, J_{gem} = 12 Hz, $J_{4',5'a}$ = 5 Hz, $C_{5'a}$H), 4.21 (dd, 1, $J_{4',5'b}$ = 5 Hz, $C_{5'b}$H), 4.61 (ddd, 1, $J_{3',4'}$ = 4 Hz, C_4·H), 5.39 (dd, 1, $J_{2',3'}$ = 1 Hz, $C_{3'}$H), 5.75 (dd, 1, $J_{1',2'}$ = 6 Hz, $C_{2'}$H), 5.98 and 6.15 (d, 1, J = 8 Hz, vinyl H's), 6.58 (d, 1, $C_{1'}$H), 6.74 (d, 1, $J_{5,6}$ = 7.5 Hz, C_5H), 8.33 (d, 1, C_6H), 9.5 and 9.9 ppm (br s, 1, NH_2). Anal. C, H, N. See Table I for other data.

Acknowledgment. We are most grateful to the staff of the Analytical Laboratories of Syntex Research for their analytical support and particularly to Dr. M. L. Maddox and Mrs. J. Nelson and to Dr. L. Tokes for their continuous help with NMR and mass spectroscopy. Thanks are also due to Dr. Tadashi Okabayashi and Toyokazu Ishigami, who have done much to coordinate the chemical synthesis at Syntex Research with the biological studies at Shionogi Research Laboratories.

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Reactions of 2-Acyloxyisobutyryl Halides with Nucleosides. 8.[1a] Synthesis and Biological Evaluation of Some 3'-Acyl and 3',5'-Diacyl Derivatives of 1-β-D-Arabinofuranosylcytosine[1b]

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Previous papers in this series have described efficient syntheses of 3'-*O*-acyl and 3',5'-di-*O*-acyl derivatives of 2,2'-anhydro-1-(β-D-arabinofuranosyl)cytosine hydrochloride (**1**, **3**). It has now been shown that the 2,2'-anhydro linkage in **1** and **3** can be selectively and efficiently cleaved by treatment with a mixture of pyridine and methanol giving the corresponding 3'-*O*-acyl and 3',5'-di-*O*-acyl derivatives of 1-β-D-arabinofuranosylcytosine (**2**, **4**). The selective hydrolysis of the more soluble derivatives can also be achieved using either aqueous pyridine or a mixture of sodium carbonate and sodium bicarbonate in aqueous dioxane. Using the above procedures 3'-*O*-acyl araC's and 3',5'-di-*O*-acyl araC's with saturated or unsaturated ester groups containing from 2 to 22 carbon atoms have been prepared, and these substances have been evaluated for cytotoxicity and antiviral activity in tissue culture and for antitumor activity against L1210 leukemia in mice. Many of the compounds show high anti-L1210 activity relative to araC itself.

1-β-D-Arabinofuranosylcytosine (araC) is a nucleoside analogue possessing substantial antileukemic,[3] anti-DNA viral,[4] and immunosuppressive[5] properties. While its clinical use against viral infections has met with mixed success,[6] araC has proved to be a valuable agent for the treatment of acute leukemias.[3] AraC, however, suffers rapid deamination and deactivation in vivo by cytidine deaminase, and its very short (12 min) half-life in man[7]

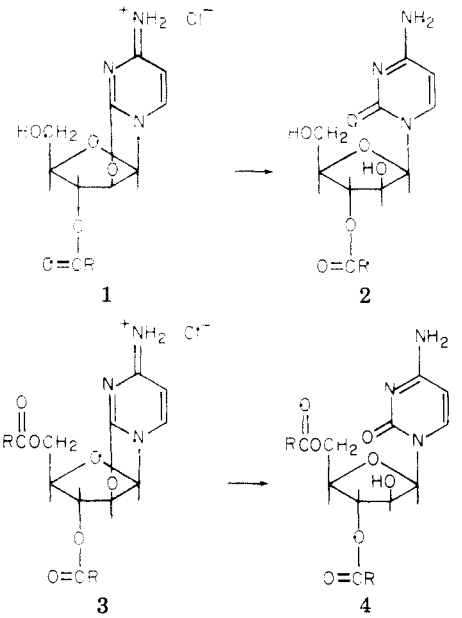
necessitates the use of complex, repetitive dosage schedules or continuous intravenous infusion.[3]

An approach to resolving the problems accompanying the short biological half-life of araC was suggested by work on the synthesis of various 5'-esters of araC.[8] Certain of these compounds, which could be prepared by direct, selective acylation of the primary 5'-hydroxyl group of araC hydrochloride,[8] were shown to have dramatically altered antileukemic activities as compared to araC itself.[8,9] In particular, certain of the less soluble 5'-esters were found to have prolonged activity against L1210 leukemia in mice since they were not substrates for cytidine deaminase and, hence, served as depot forms of araC itself.[8,9] As a result, a single administration of the drug showed the same activity as multiple injections or a continuous infusion of araC. A few 2'- and 3'-monoesters of araC have also been prepared,[10] but these syntheses have required the use of blocked intermediates followed by chromatographic separation of isomers and the yields of pure products were very low (2–5%). Montgomery and Thomas[11] have described the synthesis of the 3',5'-di-*O*-butyryl, 2',3',5'-tri-*O*-butyryl- and 2',3',5'-N^4-tetrabutyryl derivatives of araC and shown that the two former compounds showed moderate activity against L1210 leukemia while the introduction of the N^4-butyryl function led to inactivation. We have made the same observation with 2',3',5'-N^4-tetraacetyl araC. On the other hand, it has recently been reported[12] that longer chain (C_{14}–C_{22}) N^4-acyl derivatives of araC are effective agents against L1210 leukemia in mice. In the present paper we present details of our work leading to efficient syntheses of some 37 3'-*O*-acyl and 3',5'-di-*O*-acyl derivatives of araC and a survey of their cytotoxic, antiviral, and antileukemic properties. Some aspects of this work have been summarized previously,[13] and further details of the biological work will be presented at a later date.

Chemical Synthesis. In several earlier papers in this series[1a,14,15] we have described efficient specific syntheses of 2,2'-anhydro-1-(3'-*O*-acyl-β-D-arabinofuranosyl)cytosine hydrochlorides (3'-*O*-acyl cycloC's, **1**) and 2,2'-anhydro-1-(3',5'-di-*O*-acyl-β-D-arabinofuranosyl)cytosine hydrochlorides (3',5'-di-*O*-acyl cycloC's, **3**) in which the acyl groups were saturated or unsaturated fatty acids containing from 2 to 22 carbon atoms. These compounds generally exhibited cytotoxicity against HeLa cells in tissue culture and also had activity against DNA viruses and against L1210 leukemia in mice.[1a,15] The degree of antiviral and antitumor activity was, however, strongly influenced by the length and nature of the acyl groups, antiviral activity generally being maximal with C_8–C_{12} acyl groups while optimal antileukemic activity required somewhat larger acyl functions. Even modest changes in the nature of the acyl groups sometimes led to striking variations in biological response.

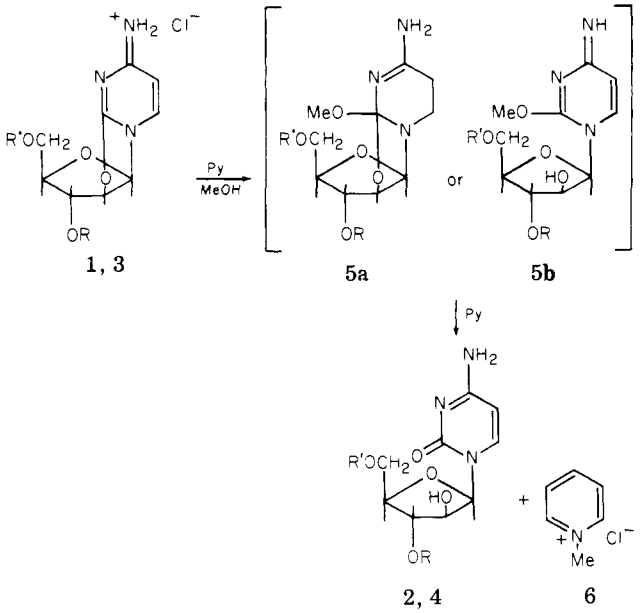
There has also been considerable recent interest in the antileukemic properties of 2,2'-anhydro-1-(β-D-arabinofuranosyl)cytosine hydrochloride (cycloC) itself (for leading references see ref 15). This compound is inert to the action of cytidine deaminase[16] but is gradually converted under physiological conditions to araC, thus providing a slow release of the latter, which is presumably the active species.[17] Our objective was to find a method for the selective cleavage of the 2,2'-anhydro linkage in **1** and **3** in order to give the corresponding 3'-*O*-acyl araC's (**2**) and 3',5'-di-*O*-acyl araC's (**4**), respectively.

It is well known that the 2,2'-anhydro linkage in cycloC is extremely stable under acidic conditions, deacetylation of 3'-*O*-acetyl cycloC with methanolic hydrogen chloride



at room temperature for several days providing a high-yield synthesis of cycloC.[14] CycloC is, however, very sensitive to alkaline hydrolysis, and Doerr and Fox[18] have shown that neutralization of cycloC hydrochloride to its free base form is accompanied by spontaneous hydrolysis to araC. It has been demonstrated in several studies that incubation of cycloC hydrochloride in buffer solutions at physiological pH's leads to a gradual cleavage to araC.[17–19] While this type of buffered hydrolysis would ideally accomplish the selective cleavage of the 2,2'-anhydro linkage without affecting the ester groups, the very low solubilities of the longer chain acyl derivatives (**1**, **3**) in both aqueous and nonaqueous solvents seriously limit this approach. In the case of the shorter chain 3'-*O*-acyl cycloC's (e.g., the 3'-*O*-octanoyl derivative **1**, R = C_7H_{15}) it was possible to obtain a dilute homogeneous solution of the nucleoside in a mixture of dioxane and aqueous carbonate buffer (pH ~9.2). After several hours at room temperature crystalline 1-(3-*O*-octanoyl-β-D-arabinofuranosyl)cytosine was isolated by chromatography on silicic acid in 55% yield.

The above method was not readily suited for use with the less soluble, higher acyl homologues of **1** and **3**. It was, however, shown that the desired selective cleavage of the 2,2'-anhydro linkage in either the 3'-monoesters **1** or the 3',5'-diesters **3** of cycloC could be achieved by heating in a 1:1 mixture of pyridine and methanol at 75–80° for 16 h. Under these conditions the higher acyl homologues are initially insoluble but dissolve giving a clear solution during the first few hours of the reaction. Completion of the hydrolysis can be judged by thin-layer chromatography using 1-butanol–acetic acid–water (5:2:3), 15–20% methanol in chloroform, or acetonitrile–0.1 M ammonium chloride (9:1), the arabinoside (**2**, **4**) in each case being less polar than the starting material (**1**, **3**). Such TLC examination showed the presence of a highly polar byproduct in each reaction, and this compound was shown by paper chromatography, paper electrophoresis, and ultraviolet spectroscopy to be *N*-methylpyridinium chloride (**6**). Since opening of the anhydronucleoside does not occur in methanol under acidic conditions, the first step is presumably formation of an equilibrium concentration of the free base followed by addition of methanol at C_2 giving either **5a** or **5b**. Subsequent dealkylation of either of these species then gives the observed 3'-*O*-acyl arabinoside and **6**. We have not made any effort to distinguish between **5a** and **5b** at this time.



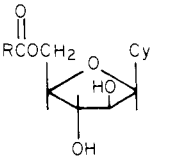
1, 3 5a 5b 2, 4 6

It is also possible to effect the cleavage of the 2,2'-anhydro linkage by heating with aqueous pyridine, this procedure having the advantage that all the by-products are volatile. While this approach was used successfully to convert 3'-*O*-acetyl cycloC (**1**, R = CH_3) into 3'-*O*-acetyl araC (**2**, R = CH_3) in a yield of 92%, the very low solubility of the higher acyl homologues of **1** and **3** makes the use of pyridine–methanol generally preferable. The latter method has been used for the preparation of most of the araC derivatives used in this work as summarized in Table I. In some cases pure crystalline esters of cycloC (**1**, **3**) were used in the pyridine–methanol reactions, while on other occasions the crude product arising from reaction of cytidine with the 2-acyloxyisobutyryl chloride[15] or from diacylation of cycloC itself[1a] was used directly. Since the latter method gives generally satisfactory overall yields of the 3'-acyl or 3',5'-diacyl araC's (**2**, **4**) starting from cytidine or cycloC, it makes these interesting compounds readily available.

In a few cases the free base forms of the acylated araC's were not readily obtained in crystalline form, although their NMR spectra indicated the presence of a single compound (e.g., **2**, R = $C_6H_5CH_2$, $CH_3(CH_2)_2CH{=}CH$; **4**, R = CH_3, C_7H_{15}, $C_6H_5CH_2$). On those occasions the compounds were isolated in the form of their readily crystalline hydrochlorides (purification method C in the Experimental Section) and the yields reported in Table I refer to crystalline products. A number of other esters were also converted to salt forms in order to examine the effect of increasing their solubility in water upon their biological properties (see later).

In order to permit a direct comparison of the biological activities of the present 3'-*O*-acyl araC's with the 5'-*O*-acyl isomers described by others,[8] we have also prepared a few of the latter compounds. Thus, using the synthetic route developed by Gish et al.[8a] araC hydrochloride was acylated using 1.1 equiv of decanoyl, palmitoyl, and behenoyl chlorides in dimethylacetamide giving the crystalline 5'-esters **7a–c** in yields of 34, 94, and 61%. Comments on the biological activities of these compounds will be found later.

All of the compounds prepared in this work have been characterized by the usual analytical and spectroscopic means. Several of the very long chain (C_{18}–C_{22}) diesters (**4**) were not sufficiently soluble to give good NMR spectra without time averaging, but with these exceptions the other



7a, R = C_9H_{19}
b, R = $C_{15}H_{31}$
c, R = $C_{21}H_{43}$

compounds gave readily interpretable spectra which clearly confirmed chemical homogeneity. The spectra of all compounds within a given series were very similar and representative examples are to be found in the Experimental Section. It was previously noted that the C_5' protons in mono- and diesters of cycloC (**1**, **3**)[1a,15] were magnetically nonequivalent. These protons in the 3'-esters of araC were, on the other hand, magnetically equivalent and appeared as doublets at 3.61 ± 0.2 ppm. As might be expected, free rotation about the C_4–C_5 bond was inhibited by 5'-ester functions, and hence the 5' protons in the diesters **4** showed magnetic nonequivalence although the spectra in that region were less well resolved due to overlapping of the C_2', C_4', and C_5 protons. As in the case of the esters of cycloC (**1**, **3**),[1a,15] mass spectroscopy was also of some diagnostic value. Even the higher diesters showed small molecular ions accompanied by stepwise fragmentation of the ester groups. As was the case with the 3'-*O*-acyl cycloC's (**1**),[15] the spectra of 3'-*O*-acyl araC's (**2**) also showed very small peaks corresponding to the molecular ions for the corresponding diesters and their acyl fragmentation products. Similarly, the spectra of completely pure diesters **4** showed minute peaks corresponding to the corresponding triesters. Since we could detect no trace of impurities in the spectral samples, we attribute these additional peaks to intermolecular thermal transesterifications taking place in the ion source. Similar transesterifications have previously been documented with other nucleoside esters.[20] Other features of the spectra were largely what would be expected from simple cytosine nucleosides and included intense peaks at m/e 112 (B + 2H) and 151 (B + 41).[21]

Biological Evaluation. The compounds described in this paper have all been examined for cytotoxicity against HeLa cells, antiviral activity against the IHD strain of vaccinia virus and the HF strain of Herpes simplex virus, and antineoplastic activity against L1210 leukemia in mice. The procedures used for these studies have been outlined in an earlier paper[15] and the results are summarized in Table I. As was the case with the various esters of cycloC,[1a,15] none of the present compounds showed any significant antibacterial or antifungal activity.

In general, all of the compounds showed rather comparable cytotoxicity against HeLa cells with the exception of the very long chain (C_{16}–C_{22}) saturated and, to a lesser degree, unsaturated diesters, which were markedly less active. This reduced activity with the long-chain diesters is similar to what we have encountered previously[1a,15] and is presumably due to the very low solubilities of those compounds in the tissue culture media. Typical of araC derivatives,[4] the various esters were inactive against RNA viruses but showed substantial activity against DNA viruses. The 3'-monoesters **2** generally showed higher activity than did the 3',5'-diesters **4**, and typically vaccinia virus was somewhat more sensitive than Herpes. As was the case with the esters of cycloC,[1a,15] the introduction of short (C_2–C_6) acyl groups led to a reduction of activity relative to the parent nucleoside (araC). In the 3'-monoester series maximal activity against vaccinia virus

Table I. Acyl Derivatives of 1-β-D-Arabinofuranosylcytosine

Ester	Formula[a]	Mol wt	Mp, °C	Yield,[b-d] %	Purifcn method[e]	Uv (MeOH)$^{H^+}$, λ_{max} (ε)	Cytotoxicity, ED_{50}, μg/ml	Antiviral ED_{50}, μM		L1210 leukemia, % ILS (30-day survivors)			
								Vaccinia	Herpes	100 mg/kg	200 mg/kg	500 mg/kg	1000 mg/kg
(1) 3′-Monoesters													
Unsubstituted	$C_9H_{13}N_3O_5$	243.22	210–212	73[c]	*l*	212 (9700), 285 (13500)	0.05	0.07	0.16	8 (0/8)	7 (0/7)	16 (0/8)	34 (0/8)
3′-Acetyl	$C_{11}H_{15}N_3O_6$	285.25	208–200	92[b]	B, EtOH–Me_2CO	212 (9400), 279 (13800)	0.14	0.58	1.45	14 (0/8)	19 (0/8)	20 (0/8)	
3′-Butyryl	$C_{13}H_{19}N_3O_6$	313.32	184–186	38[b]	A, *i*-PrOH	212 (9400), 283 (13500)	0.11	1.20	2.80		13 (0/8)	22 (0/8)	
3′-Hexanoyl	$C_{15}H_{23}N_3O_6$	341.38	181–183	77[b]	B, EtOH–Et_2O	213 (9700), 283 (14500)	0.05	0.48	1.50	5 (0/7)[k]	21 (0/7)		
3′-Octanoyl	$C_{17}H_{27}N_3O_6$	369.41	180–182	57[c]	A, BuOH	212 (10300), 283 (14600)	0.10	0.11	0.64	34 (0/8)	48 (0/8)	37 (0/8)	11 (0/8)
3′-Nonanoyl	$C_{18}H_{29}N_3O_6$	383.46	182–184	52[c]	A, B, BuOH	213 (10300), 283 (14900)	0.05	0.10	0.30		59 (0/8)	>189 (5/8)	>142 (3/8)
3′-Decanoyl	$C_{19}H_{31}N_3O_6$	397.48	183–184	67[b]	A, EtOH	213 (9600), 284 (13800)	0.09	0.10	0.34	>241 (7/8)	>224 (5/8)	>161 (5/8)	-23 (0/8)
3′-Undecanoyl	$C_{20}H_{33}N_3O_6$	411.51	179–180	44[c]	A, B, EtOH	213 (9800), 283 (14400)	0.04	0.10	0.12	>127 (2/8)	>125 (1/8)	>99 (2/8)	
3′-Dodecanoyl	$C_{21}H_{35}N_3O_6$	425.53	180–182	74[b]	A, EtOH	212 (9700), 283 (14300)	0.08	0.05	0.25	>258 (8/8)[k]	>257 (6/8)		-28 (0/8)
3′-Myristoyl	$C_{23}H_{39}N_3O_6$	453.59	174–176	78[b]	A, BuOH	212 (10500), 284 (14700)	0.08	0.18	0.64	>266 (7/8)	>284 (8/8)	-21 (0/8)	-23 (0/8)
3′-Palmitoyl	$C_{25}H_{43}N_3O_6$	481.65	193–195[f]	61[b]	A, MeOH	212 (10000), 284 (14700)	0.24	0.14	0.67	>295 (8/8)	>295 (8/8)	-20 (0/8)	-19 (0/8)
3′-Stearoyl	$C_{27}H_{47}N_3O_6$	509.70	152–154[g]	80[b]	A, EtOH	212 (10300), 283 (14200)	0.13	0.38	0.70	>219 (9/9)	>228 (5/8)		-23 (0/8)
3′-Arachidyl	$C_{29}H_{51}N_3O_6$	537.75	177–179	71[b]	B, MeOH	213 (9700), 284 (14600)	0.10	>30	>30	91 (0/8)	164 (0/8)	>274 (6/8)	>295 (8/8)
3′-Behenoyl	$C_{31}H_{55}N_3O_6$	565.80	174–175	76[b]	A, EtOH	213 (10300), 284 (14400)	1.1	>30	>30	>200 (1/8)	>215 (7/10)		>240 (8/8)
3′-Phenylacetyl	$C_{17}H_{20}N_3O_6Cl$	397.83	199–201	21[c] (HCl)	C, EtOH	284 (14700)	0.11	0.46	1.50		8 (0/6)		
3′-Dec-2-enoyl	$C_{19}H_{30}N_3O_6Cl$	431.93	193–195	17[c] (HCl)	C, EtOH	211 (21400), 282 (14700)	0.06	0.11	0.23		22 (0/7)		
3′-Undec-10-enoyl	$C_{20}H_{31}N_3O_6$	409.49	163–165	19[c]	B, EtOH	212 (9600), 283 (14300)	0.05	0.11	0.44		49 (0/8)	>191 (4/8)	>52 (2/8)
3′-Oleoyl	$C_{27}H_{45}N_3O_6$	507.68	173–175	17[c]	B, EtOH	212 (9900), 284 (14600)	0.06	0.73	0.94		67 (0/8)		
3′-Elaidoyl	$C_{27}H_{45}N_3O_6$	507.68	165–167	71[b]	B, EtOH	212 (9500), 284 (14400)	0.05	0.94	1.50	>246 (5/7)[k]	>258 (7/8)		
3′-Erucoyl	$C_{33}H_{59}N_3O_6$	563.79	163–165	71[b]	B, EtOH	213 (9700), 283 (14700)	0.16	17.5	>30	>280 (8/8)	>280 (8/8)	-34 (0/8)	-36 (0/8)

(2) 5′-Monoesters													
5′-Decanoyl	$C_{19}H_{31}N_3O_6$	397.48	126–128	34	D, EtOAc	213 (9900), 285 (13600)	0.10	0.18	0.86	59 (0/8)	>114 (2/8)	>122 (2/8)	−45
5′-Palmitoyl	$C_{25}H_{43}N_3O_6$	481.65	148–150[j]	94	D, EtOH	212 (9100), 285 (12600)	0.08	0.30	1.14	>257 (9/9)	>224 (8/9)	−40 (0/9)	
5′-Behenoyl	$C_{31}H_{55}N_3O_6$	565.80	200–201	61	D, EtOH	213 (10300), 284 (14400)	0.04	0.45	12.0	>228 (2/8)	>295 (8/8)	>295 (8/8)	45 (0/8)
(3) 3′,5′-Diesters													
3′,5′-Diacetyl	$C_{13}H_{18}N_3O_7Cl$	363.76	209–210	44[d] (HCl)	C, EtOH	211 (10300), 282 (14500)	0.06	3.2	16.0		10 (0/8)		
3′,5′-Dibutyryl	$C_{17}H_{25}N_3O_7$	383.41	171–173[h]	64[b]	B, EtOH	213 (10100), 283 (14800)	0.11	1.15	5.20	14 (0/8)	8 (0/8)		
3′,5′-Dihexanoyl	$C_{21}H_{33}N_3O_7$	439.52	Amorph	53[d]	B	213 (9700), 283 (14800)	0.07	0.22	0.90	4 (0/8)	11 (0/8)		
3′,5′-Dioctanoyl	$C_{25}H_{42}N_3O_7Cl$	532.10	153–155	60[d] (HCl)	C, EtOH	211 (10100), 283 (14500)	0.13	0.22	2.60	28 (0/8)	20 (0/8)		46 (0/8)
3′,5′-Dinonanoyl	$C_{27}H_{46}N_3O_7Cl$	560.15	Amorph	74[d] (HCl)	C, EtOH	211 (10500), 283 (15500)	0.08	0.19	0.52		71 (0/8)		
3′,5′-Didecanoyl	$C_{29}H_{49}N_3O_7$	551.74	Amorph	80[d]	B	212 (10000), 284 (14200)	0.11	0.35	0.78		>214 (5/10)	>205 (3/8)	−4 (0/8)
3′,5′-Diundecanoyl	$C_{31}H_{53}N_3O_7$	579.79	123–124	64[d]	B, EtOH	212 (9500), 283 (13900)	0.40	0.30	0.78	>280 (6/8)	>300 (8/8)	117 (0/9)	
3′,5′-Dodecanoyl	$C_{33}H_{57}N_3O_7$	607.84	120–122	81[d]	B, EtOH	211 (10300), 283 (14500)	0.50	2.05	7.20		>285 (8/8)		>260 (7/8)
3′,5′-Dimyristoyl	$C_{37}H_{65}N_3O_7$	663.95	121–123	69[d]	A, EtOH	213 (10500), 284 (15300)	1.4	5.70	10.0	>255 (9/10)	>240 (10/10)	>105 (3/8)	−12 (0/8)
3′,5′-Dipalmitoyl	$C_{41}H_{73}N_3O_7$	720.06	109–111[i]	86[b]	A, EtOH	212 (9700), 283 (14000)	>10	30	>30		>255 (7/8)		>237 (5/6)
3′,5′-Distearoyl	$C_{45}H_{81}N_3O_7$	776.17	177–179	86[b]	A, EtOH	212 (11200), 283 (15400)	10	>30	>30		124 (0/8)		>328 (8/10)
3′,5′-Diarachidyl	$C_{49}H_{89}N_3O_7$	832.28	154–155	73[d]	B, EtOH	213 (9500), 283 (14100)	>10	>30	>30	87 (0/8)	39 (0/7)		>205 (1/8)
3′,5′-Dibehenoyl	$C_{53}H_{97}N_3O_7$	888.38	110–112	63[b]	B, EtOH	213 (9500), 283 (14100)	>10	>30	>30	10 (0/6)	27 (0/6)		113 (0/6)
3′,5′-Diphenylacetyl	$C_{25}H_{26}N_3O_7Cl$	515.97	180–181	38[d] (HCl)	C, MeOH–EtOAc	283 (14300)	0.13	2.30	1.30		29 (0/8)		
3′,5′-Diundec-10-enoyl	$C_{31}H_{50}N_3O_7Cl$	612.22	142–144	47[d] (HCl)	C, EtOAc	212 (9200), 282 (13700)	0.10	0.33	0.52	>205 (4/8)[k]	>255 (7/8)		
3′,5′-Dioleoyl	$C_{45}H_{78}N_3O_7Cl$	808.61	135–137	44[d] (HCl)	C, EtOAc	210 (10900), 283 (14900)	1.10	17.5	>30	70 (0/8)[k]	>190 (3/8)		
3′,5′-Dielaidoyl	$C_{45}H_{77}N_3O_7$	772.14	134–136	49[b]	B, EtOH	211 (9900), 283 (14200)	2.50	>30	>30	14 (0/8)[k]	>253 (2/8)		
3′,5′-Dierucoyl	$C_{53}H_{93}N_3O_7$	884.35	138–140	68[b]	B, MeOH	211 (10000), 283 (14100)	>10	>30	>30	58 (0/8)	86 (0/8)		

[a] All compounds gave correct analyses for C, H, and N. [b] From purified acyl cycloC (1, 3). [c] From cytidine without purification of intermediate. [d] From cycloC without purification of intermediate. [e] Purification A, butanol–water partition and crystallization from indicated solvent; purification B, chromatography on silicic acid with 10–20% MeOH in $CHCl_3$ and crystallization; purification C, B followed by conversion to hydrochloride and crystallization; purification D, ether precipitation followed by trituration with $NaHCO_3$–H_2O and crystallization. [f] Lit.[10] mp 174–176°. [g] Lit.[10] mp 175–177°. [h] Lit.[11] mp 166–167°. [i] Lit.[8b] mp 111–112°. [j] Lit.[8a] mp 147–149°. [k] At 50 mg/kg. [l] See ref 14.

was achieved with the C_8–C_{12} acyl derivatives, all of which showed comparable effects to araC itself on a micromolar basis. Against Herpes virus maximum effectiveness was shown by 3'-undecanoyl araC. The behavior of the 3',5'-diesters was roughly comparable, maximum activity being found with the C_6–C_{11} esters, although these compounds were generally somewhat less active than were the 3'-monoesters. In both series the very long chain saturated esters showed a dramatic reduction in activity, once again almost certainly related to their lack of solubility in the tissue culture medium.

As was the case with the 3'-esters of cycloC reported previously,[15] solubilized preparations of a number of 3'-acyl araC's were also prepared and will be described at a later date. As expected, these solubilized preparations had only a modest effect on the antiviral properties of the shorter chain (up to C_{12}) esters, activities being increased by two- fivefold. On the other hand, the much less soluble longer chain derivatives showed substantially increased (10–20-fold) activities. For example, the ED_{50} values for solubilized preparations of the 3'-myristoyl and 3'-behenoyl araC derivatives were 0.01 and 3.0 μM while the nonsolubilized materials showed values of 0.18 and >30 μM, respectively.

Table I also shows the effects of the various compounds in increasing the life span of mice infected with L1210 leukemia. In each case the compound was administered as a single intraperitoneal injection 24 h after introduction of the leukemic cells. Under these conditions araC itself is a poorly effective drug, mainly due to its very short biological half life. As can be seen from Table I, the introduction of short (C_2–C_6) esters at either C_3' or at C_3' and C_5' led to only modest increases in activity relative to araC. Further increases in the size of the acyl groups, however, led to compounds showing high antileukemic activity and giving frequent long-term (30 day) survivors. As in the previous papers, the numbers in parentheses indicate the number of animals in the test group that were still alive on day 30 at the termination of the experiment.

In general, the more active 3'-*O*-acyl araC's (**2**) are somewhat more effective than are their 3'-*O*-acyl cycloC counterparts.[15] The overall patterns of activity are, however, very dependent upon the precise nature of the acyl groups. It is interesting to note, for example, that 3'-*O*-decanoyl araC (**2**, R = C_9H_{19}) is much more active at low doses than is its 3'-*O*-nonanoyl analogue **2** (R = C_8H_{17}), although it differs by only a single carbon atom. On the other hand, the decanoyl derivative showed marked toxicity at high doses (1000 mg/kg) while this was not significant in the nonanoyl homologue. A similar striking difference is to be seen in comparing the 3'-stearoyl (C_{18}) and 3'-arachidyl (C_{20}) esters of araC, the former showing considerably greater activity at low doses while the latter is highly effective at the highest doses tested.

In the 3',5'-diester series the intermediate chain length diesters (C_{10}–C_{14}) show quite high activity at the lower doses while some toxicity is apparent at higher doses. On the other hand, the longer chain homologues (C_{16}–C_{20}) are highly effective at high doses and show little toxicity. A number of the unsaturated esters in both the mono- and diester series showed high activity at low doses.

In view of the low solubilities of many of the compounds in aqueous media, we have prepared salts of several representative examples. Most frequently we have prepared the readily crystalline hydrochlorides, but in some cases we have also studied nitrate, sulfate, phosphate, oxalate, succinate, and citrate salts and have compared the biological activities of these salt forms with those of the free bases. In most cases the salts showed HeLa cell cytotoxicities very close to those of the free bases. Similarly, the antiviral activities of most of the salts examined were roughly equal to, or slightly less than (perhaps one-half or one-third), those of the corresponding free base. A notable exception was the hydrochloride of 3'-erucoyl araC, which showed ED_{50} values of 0.38 and 1.20 μM against vaccinia and Herpes viruses while the comparable values for the free base were 17.5 and >30 μM. This suggests that the low activity of the free base was, indeed, due to its very low solubility in the tissue culture medium and that this effect was counteracted by forming the somewhat more soluble salt. Increased solubility did not, however, appear to have any beneficial effect upon in vivo activity against L1210 leukemia. In each case we have examined, the activity of the salt was somewhat lower than that of the free base, particularly with the shorter chain esters. In the case of the long-chain 3'-erucoyl araC hydrochloride, the salt showed essentially the same high activity as the free base, but in no case was one of the salts found to be superior. It would appear that absolute solubility is not in itself the sole limiting factor in the maintenance of effective in vivo drug levels.

Finally, it was of interest to directly compare the biological activities of several 3'-esters of araC with their 5'-*O*-acyl counterparts. As was mentioned earlier, very low yield syntheses of the 2'-*O*- and 3'-*O*-benzoyl, palmitoyl, and stearoyl derivatives of araC have been previously reported[10] and it has been stated that the biological activities of these compounds are not comparable to the 5'-esters.[8b] We have prepared the 5'-*O*-decanoyl, palmitoyl, and behenoyl derivatives of araC (**7a–c**) and their activities are outlined in Table I.

It can be seen from Table I that with respect to antiviral activity the 3'-decanoyl and palmitoyl esters are roughly twice as active as their 5'-counterparts against both vaccinia and Herpes viruses. The C_5-behenoyl ester is, however, distinctly more antiviral than the C_3-ester, probably suggesting that it has greater solubility in the tissue culture medium. In support of this, a solubilized preparation of 3'-behenoyl araC shows a tenfold increase in activity while a comparable solubilization of the 5'-ester leads to only a twofold increase. With respect to anti-L1210 activity, careful dose–response curves have been determined for both series of compounds and chemotherapeutic indices (CI, defined as the ratio of the dose producing maximum % ILS to the dose giving 30% ILS) have been determined. For the 3'-esters the figures for ILS_{30}, ILS_{max}, and CI were as follows: 3'-decanoyl araC (5 mg/kg, 150 mg/kg, 30), 3'-palmitoyl araC (1, 200, 200), 3'-behenoyl araC (2, 2000, 1000). Corresponding figures for the 5'-esters were: 5'-decanoyl araC (20, 500, 25), 5'-palmitoyl araC (2, 150, 75), and 5'-behenoyl araC (1, 500, 500). From the above it can be concluded that in each of the above cases the chemotherapeutic index of the 3'-ester is superior to that of its 5'-counterpart.

The synthetic methods described in this paper and in its precursors[1a,15] make a wide range of 3'-esters and 3',5'-diesters of cycloC and araC readily available. These compounds show an interesting range of biological activities and provide interesting series for structure–activity relationships. In future papers we will consider the effects of different dosage regimens and modes of administration as well as the results of tests with other tumor systems.

Experimental Section

General Methods. The general methods used are outlined in a previous paper.[15]

General Synthetic Methods Used for Preparation of 2 and 4. 1-(3-*O*-Myristoyl-β-D-arabinofuranosyl)cystosine (2, R = $C_{13}H_{27}$) (Purification Method A). A suspension of 2,2'-anhydro-1-(3'-*O*-myristoyl-β-D-arabinofuranosyl)cytosine hydrochloride (1, R = $C_{13}H_{27}$, 22.0 g, 46.6 mmol)[15] in a mixture of pyridine (110 ml) and methanol (110 ml) was stirred at 80° for 16 h. An evaporated aliquot of the clear solution was examined by TLC using 1-butanol–acetic acid–water (5:2:3) which showed the starting material to be gone with formation of a new, somewhat less polar spot and a spot near the origin. The solvents were evaporated in vacuo and the residue was coevaporated with methanol. The oily residue was dissolved in warm 1-butanol (350 ml) and washed twice with water (250 ml). The butanol solution was dried ($MgSO_4$) and concentrated in vacuo until crystallization started. The solid was redissolved and cooled giving 16.5 g (78%) of pure **2** (R = $C_{13}H_{27}$) with mp 174–176°. Alternatively, the product could be readily crystallized from ethanol: NMR (Me_2SO-d_6) 0.83 (t, 3, CH_3), 1.24 (s, 20, CH_2's), 1.53 (m, 2, $COCH_2CH_2$), 2.33 (t, 2, $COCH_2$), 3.61 (d, 1, $J_{4',5'}$ = 5 Hz, $C_5'H_2$), 3.88 (dt, $J_{3',4'}$ = 2 Hz, $C_4'H$) 4.06 (m, 1, $C_2'H$), 4.95 (dd, 1, $J_{2',3'}$ = 2 Hz, $C_3'H$), 5.69 (d, 1, $J_{5,6}$ = 7.5 Hz, C_5H), 5.96 (d, 1, $J_{1',2'}$ = 3.5 Hz, $C_1'H$), 7.15 (br s, 2, NH_2), 7.61 ppm (d, 1, C_6H); mass spectrum (70 eV) *m/e* 453 (M^+), 242 (M^+ – RCO), 226 (R – RCO_2), 663 (small, diester). Anal. C, H, N. See Table I for other data.

A sample of **2** (R = $C_{13}H_{27}$, 2.27 g, 5 mmol) was dissolved in methanol (50 ml) and methanolic hydrogen chloride (5.5 mmol) was added. After evaporation of the solvent the residue was crystallized from ethanol giving 1.79 g (73%) of the hydrochloride with mp 179–181°: λ_{max} (MeOH) 213 nm (ε 9200), 283 (13700). Anal. C, H, N.

1-(3-*O*-Arachidyl-β-D-arabinofuranosyl)cytosine (2, R = $C_{19}H_{39}$) (Purification Method B). (a) From Purified 1 (R = $C_{19}H_{39}$). A suspension of pure **1** (R = $C_{19}H_{39}$, 3.0 g, 5.4 mmol) in pyridine (50 ml) and methanol (50 ml) was stirred overnight at 80° leading to complete reaction as judged by TLC using 1-butanol–acetic acid–water (5:2:3). The solvents were evaporated in vacuo and a solution of the oily residue in chloroform was applied to a column containing 200 g of silicic acid. The column was eluted with chloroform containing 5, 10, and 15% methanol, the fractions being examined by TLC using chloroform–methanol (4:1). Evaporation of the pooled product fractions followed by crystallization from methanol gave 2.04 g (71%) of **2** (R = $C_{19}H_{39}$) with mp 177–179°. An analytical sample had mp 177.5–178.5°: NMR (Me_2SO-d_6) 0.84 (t, 3, CH_3), 1.24 (s, 32, CH_2's), 1.5 (m, 2, $COCH_2CH_2$), 2.34 (t, 2, CH_2), 3.63 (d with D_2O, 2, $J_{4',5'}$ = 5 Hz, $C_5'H_2$), 3.89 (dt, 1, $J_{3',4'}$ = 2 Hz, $C_4'H$), 4.06 (m, 1, $C_2'H$), 4.94 (dd, 1, $J_{2',3'}$ = 1.5 Hz, $C_3'H$), 5.67 (d, 1, $J_{5,6}$ = 7.5 Hz, C_5H), 5.96 (d, 1, $J_{1',2'}$ = 3.5 Hz, $C_1'H$), 7.08 (br s, 2, NH_2), 7.60 ppm (d, 1, C_6H); mass spectrum (70 eV) *m/e* 537 (M^+), 427 (M^+ – cytosine), 295 (M^+ – RCO), 151 (base + 41),[21] 112 (base + 2H). Anal. C, H, N. See Table I for other data.

(b) Without Purification of 1. Cytidine (13.6 g, 56 mmol) was added to a solution of crude 2-arachidoyloxyisobutyryl chloride (223 mmol)[15] in acetonitrile (280 ml) and the resulting suspension was stirred at 60° for 16 h at which point TLC using 1-butanol–acetic acid–water (5:2:3) showed the reaction to be complete. Most of the acetonitrile was removed in vacuo and the residue was added to ether (1 l.) giving crude **1** (R = $C_{19}H_{39}$, 30.3 g) as a white solid that was collected, washed with ether, and dried in vacuo. This material was suspended in pyridine (500 ml) and methanol (500 ml) and stirred at 80° for 16 h. The solvents were evaporated in vacuo and the residue was chromatographed on a column of silicic acid (1 kg) as in (a) above. Crystallization of the pooled product fractions from ethanol gave 16.28 g (54% from cytidine) of **2** (R = $C_{19}H_{39}$) identical with that above.

1-(3,5-Di-*O*-octanoyl-β-D-arabinofuranosyl)cytosine Hydrochloride (4, R = C_7H_{15}) (Purification Method C). A mixture of 2,2'-anhydro-1-(β-D-arabinofuranosyl)cytosine hydrochloride (2.62 g, 10 mmol) and octanoyl chloride (6.48 g, 40 mmol) in dimethylacetamide (50 ml) was stirred at 37° for 16 h at which point TLC using acetonitrile–0.1 M ammonium chloride (9:1) showed the reaction to be complete. Ether (500 ml) was added and the precipitate was collected by centrifugation, washed with ether, and dried in vacuo giving 6.8 g of crude **3** (R = C_7H_{15}). This material was directly stirred at 80° for 16 h with pyridine (100 ml) and methanol (100 ml). The solvents were evaporated and the residue was chromatographed on a column of silicic acid (500 g) using 5 and 10% methanol in chloroform. The pure product fractions were evaporated leaving 4.21 g (85%) of TLC and NMR homogeneous product that could not be obtained in crystalline form. It was dissolved in methanol and after addition of methanolic hydrogen chloride (9.5 mmol) the solvent was evaporated and the residue was crystallized from ethyl acetate giving 3.19 g (60% from cycloC) of **4** (R = C_7H_{15}) with mp 153–155°: NMR (Me_2SO-d_6) 0.86 (t, 6, CH_3), 1.25 (s, 16, CH_2's), 1.5 (m, 4, $COCH_2CH_2$), 2.31 (t, 4, $COCH_2$), 4.2 (m, 4, $C_2'H$, $C_4'H$, $C_5'H_2$), 5.00 (m, 1, $C_3'H$), 6.02 (d, 1, $J_{1',2'}$ = 3.5 Hz, $C_1'H$), 6.21 (d, 1, $J_{5,6}$ = 7.5 Hz, C_5H), 7.87 (d, 1, C_6H), 8.90 and 9.91 ppm (br s, 1, NH_2). The spectrum of the free base in $CDCl_3$ was similar except that the C_5' protons were then well resolved from $C_2'H$ and $C_4'H$ and appeared at 4.20 (dd, 1, J_{gem} = 12 Hz, $J_{4',5'a}$ = 5 Hz, $C_{5'a}H$) and 4.32 ppm (dd, 1, $J_{4',5'b}$ = 3.5 Hz, $C_{5'b}H$). The C_5H and C_6H signals also showed the expected shifts to 5.66 and 7.49 ppm, respectively; mass spectrum (free base, 70 eV) *m/e* 621 (small, triester), 495 (M^+), 477 ($M^+ - H_2O$), 385 (M^+ – base), 368 (M^+ – RCO), 352 ($M^+ - RCO_2$), 344 (*m/e* 352 – H_2O), 151 (base + 41),[21] 127 (RCO), 112 (B + 2H). Anal. C, H, N. See Table I for other data.

1-(3-*O*-Acetyl-β-D-arabinofuranosyl)cytosine (2, R = CH_3). (a) Using Aqueous Pyridine. A solution of pure **1** (R = Me, 1.0 g, 3.3 mmol) in water (7.5 ml) and pyridine (15 ml) was heated at 60° for 20 h and then evaporated to dryness. The residue was coevaporated several times in vacuo with methanol–chloroform (1:1) and then crystallized from ethanol–acetone giving 684 mg (73%) of **2** (R = CH_3) with mp 208–210°. Preparative TLC of the mother liquors using chloroform–methanol (7:2) gave a further 182 mg (total yield 92%) of crystalline product: NMR (Me_2SO-d_6) 2.08 (s, 3, OAc), 3.63 (d, 2, $J_{4',5'}$ = 5.5 Hz, $C_5'H_2$), 4.00 (dt, $J_{3',4'}$ = 2.5 Hz, $C_4'H$), 4.20 (dd, 1, $J_{1',2'}$ = 4 Hz, $J_{2',3'}$ = 2 Hz, $C_2'H$), 4.97 (dd, 1, $C_3'H$), 5.94 (d, 1, $C_1'H$), 6.18 (d, 1, $J_{5,6}$ = 8 Hz, C_5H), 8.00 (d, 1, C_6H), 8.82 and 9.83 ppm (br s, 1, NH_2). Anal. C, H, N. See Table I for other data.

(b) Using Pyridine–Methanol. A solution of **1** (R = CH_3, 3.59 g, 11.8 mmol) in methanol (50 ml) and pyridine (50 ml) was heated at 80° for 16 h and then evaporated to dryness. The residue was coevaporated twice with methanol–chloroform (1:1) and crystallized from ethanol–acetone giving 2.15 g (64%) of **2** (R = CH_3) identical with that from (a) above. The mother liquors were not further purified.

1-(3-*O*-Octanoyl-β-D-arabinofuranosyl)cytosine (2, R = C_7H_{15}). (a) Using Pyridine–Methanol. Cytidine (243.8 g, 1 mol) was added to a solution of 2-octanoyloxyisobutyryl chloride (622 g, 2.5 mol) and the mixture was stirred at 37° for 3 h. The mixture was then filtered and the white solid was washed with ether and dried in vacuo giving 216 g of **1** (R = C_7H_{15}) that was homogeneous by TLC using 1-butanol–acetic acid–water (5:2:3). This material was added to pyridine (2 l.) and methanol (2 l.) and stirred at 80° for 16 h. Examination by TLC showed the hydrolysis to be incomplete and the reaction was continued for a further 24 h before evaporation to dryness. The residue was coevaporated twice with methanol (250 ml), dissolved in warm 1-butanol (500 ml), and washed twice with saturated aqueous sodium chloride (250 ml), dried ($MgSO_4$), and somewhat concentrated in vacuo. The resulting crystals were washed with butanol and ether and dried in vacuo giving 118 g (57% from cytidine) of **2** (R = C_7H_{15}) in three crops with mp 180–182°. Further product could be isolated from the mother liquors by chromatography on a column of silicic acid (ten parts by weight) using 5, 10, and 15% methanol in chloroform: NMR (Me_2SO-d_6) 0.86 (t, 3, CH_3), 1.25 (s, 8, CH_2's), 1.5 (m, 2, $COCH_2CH_2$), 2.34 (t, 2, $COCH_2$), 3.60 (d, 1, $J_{4',5'}$ = 5.5 Hz, $C_5'H_2$), 3.88 (dt, 1, $J_{3',4'}$ = 2 Hz, $C_4'H$), 4.04 (dd, 1, $J_{1',2'}$ = 3.5 Hz, $J_{2',3'}$ = 2 Hz, $C_2'H$), 4.94 (dd, 1, $C_3'H$), 5.67 (d, 1, $J_{5,6}$ = 7.5 Hz, C_5H), 5.96 (d, 1, $C_1'H$), 7.56 ppm (d, 1, C_6H). Anal. C, H, N. See Table I for further data.

(b) Using Sodium Carbonate–Sodium Bicarbonate. A solution of **1** (R = C_7H_{15}, 388 mg, 1 mmol) in water (15 ml) and dioxane (10 ml) containing sodium carbonate (150 mg) and sodium bicarbonate (200 mg) was kept at room temperature for 2 h and then evaporated to dryness. The residue was coevaporated twice with ethanol and then extracted three times with hot ethanol (10 ml). The extracts were evaporated and purified by preparative TLC using chloroform–methanol (3:1) and crystallized from

ethanol giving 204 mg (55%) of **2** (R = C_7H_{15}) identical with that from (a) above.

1-(5-*O*-Behenoyl-β-D-arabinofuranosyl)cytosine (7c) (Purification Method D). A mixture of araC hydrochloride (6.46 g, 23 mmol) and behenoyl chloride (from 10 g, 29 mmol, of behenic acid and 14 g of thionyl chloride at 50° for 2 h followed by evacuation at 50° under high vacuum) in dimethylacetamide (125 ml) was stirred at 40° for 16 h. The solvent was then evaporated in vacuo and the residue was dissolved in hot ethyl acetate (125 ml) and filtered. Addition of ether (125 ml) to the filtrate gave a white precipitate that was collected and stirred with saturated aqueous sodium bicarbonate (200 ml) until gas evolution ceased. The precipitate was then collected, washed with water, and dried giving 12.1 g of crude product. Crystallization from ethanol gave 10.62 g (81%) of **7c** that was contaminated with a trace of a highly nonpolar product that could not be removed by recrystallization. Accordingly, the product was dissolved in hot methanol, added to silicic acid (100 g), and evaporated to dryness. The residue was added to the top of a column containing fresh silicic acid (1 kg) packed in chloroform and the column was eluted with chloroform–methanol (19:1) to remove the impurity and then with 4:1 to remove the product. Crystallization from methanol then gave 8.01 g (61%) of pure **7c** with mp 200–201°: too insoluble for NMR.

Acknowledgment. We are most grateful to Drs. Tadashi Okabayashi and Toyokazu Ishigami, who have done much to coordinate the chemical syntheses performed at Syntex Research with the biological studies from Shionogi Research Laboratories.

References and Notes

(1) (a) For paper 7, see E. K. Hamamura, M. Prystasz, J. P. H. Verheyden, J. G. Moffatt, K. Yamaguchi, N. Uchida, K. Sato, A. Nomura, O. Shiratori, S. Takase, and K. Katagiri, *J. Med. Chem.*, preceding paper in this issue. (b) Contribution No. 120 from the Institute of Molecular Biology, Syntex Research, Palo Alto, Calif. 94304.
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