Reactions of 2-Acyloxyisobutyryl Halides with Nucleosides. 7.[1a] Synthesis and Biological Evaluation of Some 2,2'-Anhydro-1-(3',5'-di-*O*-acyl-β-D-arabinofuranosyl)cytosine Hydrochlorides[1b]

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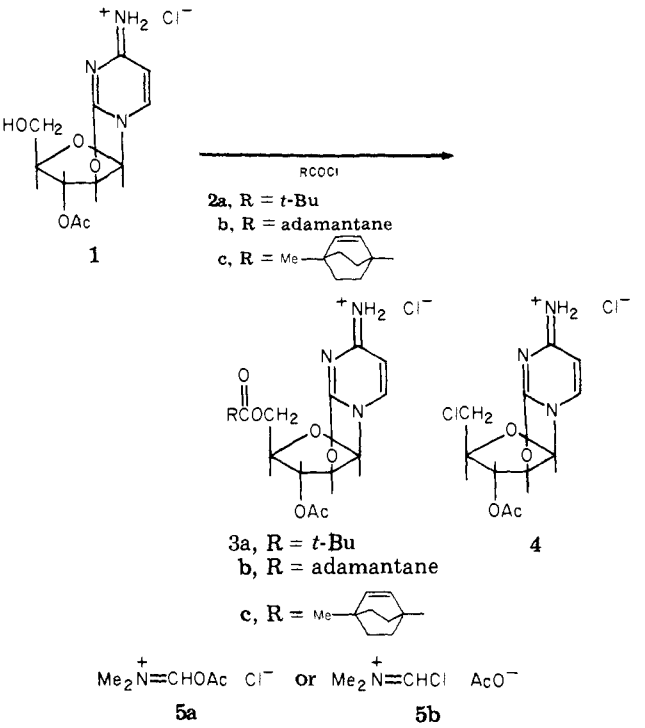
The direct acylation of 2,2'-anhydro-1-(β-D-arabinofuranosyl)cytosine hydrochloride (cycloC) with a homologous series of saturated and unsaturated acyl chlorides in dimethylacetamide has been investigated. Such acylation reactions have made available a considerable number of 3',5'-diesters of cycloC that have been examined for biological activities. The compounds all show cytotoxicity against HeLa cells in tissue culture and, with the exception of the highly insoluble long-chain diesters (C_{16}–C_{22}), show pronounced activity against vaccinia and Herpes simplex viruses. Against L1210 leukemia in mice the compounds show varied activities, the C_{12}–C_{14} saturated diesters and the C_{18}–C_{22} unsaturated diesters being highly effective. Other diesters, varying by only a few methylene groups, show dramatically different results.

In the preceding paper in this series we have shown that the reactions of cytidine with a series of saturated or unsaturated 2-acyloxyisobutyryl chlorides provide a useful, general synthesis of 2,2'-anhydro-1-(3'-*O*-acyl-β-D-arabinofuranosyl)cytosine hydrochlorides.[1a] These compounds are selective 3'-esters of 2,2'-anhydro-1-(β-D-arabinofuranosyl)cytosine hydrochloride (cycloC), a substance of current clinical interest as an antitumor agent.[3] A general introduction to this subject has been previously presented.[1a] Since the size of the acyl group was varied so as to contain from 2 to 22 carbon atoms in either a saturated or unsaturated array, the physical properties of the molecules were also diverse, the lower members of the series being freely water soluble while the largest esters had very low solubilities. The biological activities of these compounds, and in particular the antitumor activity, also varied widely, the larger esters showing strong action of long duration against L1210 leukemia in mice. In order to further extend the scope of our biological studies using compounds of even lower polarity we decided to investigate the synthesis of a variety of 3',5'-di-*O*-acyl derivatives of cycloC. Some aspects of this work have previously been briefly reviewed.[4]

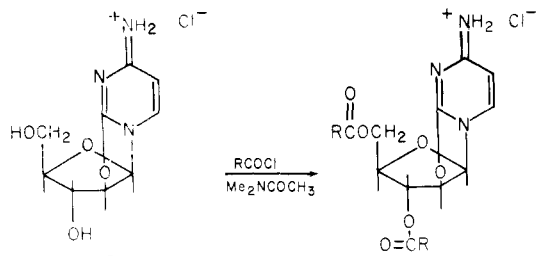
Chemical Synthesis. Since 2,2'-anhydro-1-(3'-*O*-acetyl-β-D-arabinofuranosyl)cytosine hydrochloride (**1**) was a particularly readily available compound,[5] some of our earlier syntheses used this substance as a starting material. The very low solubilities of **1**, and of cycloC itself, in most chemically inert organic solvents, and the ease with which the 2,2'-anhydro ring in cycloC derivatives is opened under mildly basic conditions in either aqueous[6] or nonaqueous[7] media, severely limit the conditions that can be employed for acylation. Our initial efforts involved the attempted acylation of **1** with pivaloyl chloride (**2a**) or with adamantoyl chloride (**2b**) in dimethylformamide (DMF) without added base. These reactions were slow and required 6–12 days at room temperature to reach completion. After this time the crude products were precipitated with ether and crystallized from acetonitrile. In each case, examination of the product by NMR spectroscopy showed it to be a mixture of the desired 2,2'-anhydro-1-(3'-*O*-acetyl-5'-*O*-acyl-β-D-arabinofuranosyl)cytosine hydrochloride (**3a,b**) and the previously described 2,2'-anhydro-1-(3'-*O*-acetyl-5'-chloro-5'-deoxy-β-D-arabinofuranosyl)cytosine hydrochloride (**4**)[5] in roughly equal amounts. The expected close doubling of the signals due to $C_{1'}H$, $C_{2'}H$, and $C_{6'}H$ was apparent while the $C_{5'}$ protons were well separated, those arising from **4** being roughly 0.3 ppm upfield of those from **3**. The chlorination reaction must be a consequence of a side reaction between the acid chloride and DMF forming a Vilsmeier–Haack type of complex such as **5**. Such complexes, usually prepared from DMF and phosphorus oxychloride, thionyl chloride, or phosgene,[8] are also formed from acyl halides[9] and have been shown to function as halogenating agents.[9b,10]



By conducting the acylation of **1** with acid chlorides **2** in dimethylacetamide rather than DMF the halogenation reaction was avoided. In this way the 3'-*O*-acetyl-5'-*O*-acyl derivatives **3a–c** of cycloC were prepared in high yields. It was also of interest to directly diacylate 2,2'-anhydro-1-(β-D-arabinofuranosyl)cytosine hydrochloride (cycloC, **6**) itself and, in view of the results above, this was examined using an excess (generally 4 molar equiv) of the acyl chloride in dimethylacetamide (roughly 4 ml/mmol of nucleoside). Since neither cycloC (**6**) itself nor the higher 3',5'-di-*O*-acyl derivatives **7** are soluble in dimethylacet-

amide, a clear solution usually does not result except with the shorter chain esters. The appearance of the suspension changes noticeably, however, and generally after 16–48 h at 37° TLC shows the complete absence of cycloC. In the case of the very long chain esters (arachidyl, behenoyl, erucoyl) it is advantageous to run the acylation at 50–65° in order to obtain complete reaction.

Once the reaction is complete, simple filtration frequently leads to the isolation of reasonably pure 3',5'-di-*O*-acyl derivatives 7 in moderate yield. The addition of ether to the reaction mixture, however, leads to the recovery of high yields of 7 without contamination by excess acyl chloride or acid. Under these conditions the product is contaminated by variable amounts of nonultraviolet-absorbing Vilsmeier–Haack type complexes arising from the reaction of dimethylacetamide with the acyl chloride. These substances are extremely soluble in lower alcohols and crystallization of the crude products from methanol or ethanol readily provides the pure diesters 7 in satisfactory yields. We have generally not examined the by-products arising from dimethylacetamide, but in several cases we have isolated a crystalline compound from the mother liquors, the NMR spectrum of which shows only six-proton and three-proton singlets at 3.23 and 2.61 ppm, respectively, in $CDCl_3$. While this compound is very hygroscopic and does not give a totally satisfactory elemental analysis, its melting point is very close to that described for 1-chloroethylidenedimethylammonium chloride (8) by Bosshard and Zollinger.[8] Such a compound could arise via initial O-acylation of dimethylacetamide followed by addition of chloride ion and elimination of the acyloxy anion.



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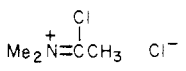

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Following reaction conducted as described above the isolation by crystallization of pure diesters 7 usually presents no problems. Occasionally minor amounts of more polar by-products with the typical ultraviolet spectra of 6 or 7 are to be found in the mother liquors, and in the case of a reaction using stearoyl chloride such a by-product was isolated in crystalline form. Interestingly enough, this product proved to be identical (NMR) to the 3'-*O*-stearoyl cycloC that we have described previously rather than being the 5'-monoester that would be expected from only partial acylation. It seems more likely that the monoester arose from selective partial loss of the 5'-*O*-acyl substitutent from the diester 7 during crystallization from methanol. The low solubilities of the higher esters required the use of substantial amounts of methanol and considerable heating in order to obtain a clear solution. The lability of the ester groups toward alcohols was quite apparent when dealing with the short-chain homologues and attempted crystallization of, e.g., the dihexanoyl ester 7 (R = C_5H_{11}) from methanol consistently led to partial decomposition. By utilizing crystallization from acetonitrile or 2-propanol this problem could be avoided. Instability did not appear to be a problem with the longer chain length esters and details of the methods used for preparation of the various compounds of type 7 are outlined in Table I. The only compound that could not be purified by direct crystallization was the dihexa-2,4-dienoyl ester 7 (R = CH=CHCH=CHCH$_3$). A number of by-products accompanied its preparation and isolation of crystalline product could only be achieved in low yield following chromatography on silicic acid.

All of the diesters that have been prepared in this work have been characterized as crystalline hydrochlorides giving correct elemental analyses for C, H, and N. All of the derivatives gave ultraviolet spectra typical of cycloC (see Table I). The long-chain (C_{16}–C_{22}) saturated diesters were not sufficiently soluble, even in Me_2SO-d_6, to give satisfactory NMR spectra. The other derivatives, however, all gave spectra that were extremely similar to one another and are outlined in the selected examples to be found in the Experimental Section. As expected, the patterns of the sugar and base protons were very similar to those of the related 3'-*O*-acyl cycloC's[1a] with the exception that the $C_{5'}$ protons were deshielded by 0.6–0.7 ppm and $C_{4'}$ H by 0.2 ppm. As in the case of other O^2,2'-cyclocytidine derivatives, the $C_{5'}$ protons were magnetically nonequivalent. The compounds in general gave satisfactory mass spectra, with even the long-chain diesters showing small but significant molecular ions corresponding to the free base form.

It may be noted that the preparation in 40% yield of 2,2'-anhydro-1-(3',5'-di-*O*-acetyl-β-D-arabinofuranosyl)-cytosine hydrobromide has recently been described by Marumoto and Honjo[11] via reaction of cytidine with acetyl bromide in acetonitrile under reflux. Extension of this method to higher homologues seems to offer some difficulties, however, since the comparable reaction using isobutyryl bromide gave the analogous anhydronucleoside diester in only 11% yield.

Biological Testing. The various compounds described in this paper have been examined for cytotoxicity against HeLa cells, antiviral activity in tissue culture, and antitumor activity against L1210 leukemia in mice following the general protocols outlined in the previous paper in this series.[1a] The compounds showed general cytotoxicity against HeLa cells in tissue culture, but the variability was somewhat more pronounced than was observed with the 3'-*O*-monoesters of cycloC,[1a] the long-chain esters (C_{16}–C_{22}) being markedly less active than the lower homologues.

As in the case of the 3'-monoesters of cycloC,[1a] the diesters showed little, if any, antibacterial and antifungal action but had pronounced activity against DNA viruses such as vaccinia and Herpes simplex. They were not active against RNA viruses such as polio, measles, or influenza. As can be seen in Table I, vaccinia virus was generally somewhat more sensitive than Herpes simplex and maximum activity was found with the medium-sized saturated or unsaturated esters (C_9–C_{11}), which showed values of ED_{50} comparable or slightly lower than that of cycloC itself. As the chain length was increased beyond C_{12}, however, the activity fell off quite sharply, the long-chain saturated esters being essentially ineffective. As in the case of the 3'-monoesters of cycloC,[1a] the reduced antiviral activities of the long-chain esters are probably due to the very low solubilities of those compounds in aqueous media. Since the solubilities of the 3',5'-diesters are considerably lower than those of the 3'-monoesters, the major fall-off in activity occurs at somewhat shorter chain lengths and is fully apparent with the 3',5'-dipalmitate 7 (R = $C_{15}H_{31}$). In general, the unsaturated esters appear

Table I. 3′,5′-Diacyl Derivatives of 2,2′-Anhydro-1-(β-D-arabinofuranosyl)cytosine Hydrochloride (7)

Structure	Formula	Mol wt	Mp, °C	Yield, %	Rxn conditions	Purifcn method[b]	Uv (MeOH)$^{H^+}$, λ_{max} (ε)	Cytotoxicity, ED_{50}, μg/ml	Antiviral ED_{50}, μM		L1210 leukemia, % ILS (30-day survivors)			
									Vaccinia	Herpes	50 mg/kg	200 mg/kg	500 mg/kg	1000 mg/kg
(a) Saturated														
Unsubstituted (cycloC)	$C_9H_{12}N_3O_4Cl$	261.68	262–264	73[a]		B, MeOH–Me_2CO	232 (9600), 262 (11 000)	0.06	0.41	0.70		25 (0/8)	67 (0/8)	89 (0/8)
3′,5′-Diacetyl	$C_{13}H_{16}N_3O_6Cl$	345.74	216–218	70	37°, 16 h	A, MeCN	234 (9600), 263 (11 000)	0.20	0.69	2.40		7 (0/8)		
3′,5′-Dibutyryl	$C_{17}H_{24}N_3O_6Cl$	401.86	232–233	76	37°, 16 h	A, *i*-PrOH	234 (10 200), 263 (11 600)	0.11	1.55	4.90		58 (0/10)	85 (0/10)	−100 (0/8)
3′,5′-Dihexanoyl	$C_{21}H_{32}N_3O_6Cl$	457.97	201–202	56	37°, 16 h	A, MeCN	234 (9800), 263 (10 800)	0.11	0.27	1.00	26 (0/8)	−61 (0/8)	−86 (0/8)	−100 (0/7)
3′,5′-Dioctanoyl	$C_{25}H_{40}N_3O_6Cl$	514.08	211–213	63	37°, 16 h	A, EtOH	232 (9900), 262 (11 000)	0.10	0.56	1.35		28 (0/8)		
3′,5′-Dinonanoyl	$C_{27}H_{44}N_3O_6Cl$	542.13	209–210	43	37°, 16 h	A, MeOH	233 (10 000), 263 (11 200)	0.06	0.32	0.42	14 (0/8)	−88 (0/8)		
3′,5′-Didecanoyl	$C_{29}H_{48}N_3O_6Cl$	570.18	206–208	69	50°, 16 h	A, *i*-PrOH	234 (10 700), 264 (12 200)	0.20	0.32	1.10	14 (0/10)	−69 (0/8)		−72 (0/8)
3′,5′-Diundecanoyl	$C_{31}H_{52}N_3O_6Cl$	598.24	204–205	42	50°, 16 h	A, EtOH	234 (9900), 264 (10 900)	0.32	0.41	0.66	88 (0/8)	>179 (3/8)	>20 (1/8)	
3′,5′-Didodecanoyl	$C_{33}H_{56}N_3O_6Cl$	626.29	201–203	68	37°, 16 h	A, MeOH	234 (9900), 262 (11 300)	0.48	0.70	3.45	>195 (4/8)	>310 (8/8)	−23 (0/8)	−18 (0/8)
3′,5′-Dimyristoyl	$C_{37}H_{64}N_3O_6Cl$	682.40	195–197	80	40°, 48 h	A, MeOH	236 (9900), 262 (11 600)	0.48	1.75	6.05	>129 (2/9)	>295 (5/7)	−20 (0/8)	−18 (0/8)
3′,5′-Dipalmitoyl	$C_{41}H_{72}N_3O_6Cl$	738.51	194–197	84	37°, 72 h	A, MeOH	235 (10 800), 264 (12 400)	4.0	28	>30		231 (0/8)		
3′,5′-Distearoyl	$C_{45}H_{80}N_3O_6Cl$	794.62	192–193	83	37°, 48 h	A, MeOH	235 (10 700), 264 (12 200)	3.8	>30	>30	68 (0/8)	125 (0/8)		>255 (6/6)
3′,5′-Diarachidyl	$C_{49}H_{88}N_3O_6Cl$	850.72	202–203	94	50°, 20 h	A, MeOH	235 (10 800), 263 (11 700)	2.5	>30	>30	8 (0/8)	31 (0/7)		107 (0/8)
3′,5′-Dibehenoyl	$C_{53}H_{96}N_3O_6Cl$	906.83	179–180	59	65°, 16 h	A, MeOH	235 (10 700), 263 (12 300)	>10	>30	>30	15 (0/8)	55 (0/9)		139 (0/10)
3′-Acetyl-5′-pivaloyl	$C_{16}H_{22}N_3O_6Cl$	387.82	242–246	71	20°, 14 days	A, MeOH	235 (10 000), 262 (11 800)	0.20	0.77	3.20		18 (0/8)		
3′-Acetyl-5′-adamantoyl	$C_{22}H_{28}N_3O_6Cl$	465.92	221–224	87	20°, 26 days	A, $CHCl_3$–EtOH	235 (11 300), 262 (12 400)	0.20	0.64	2.10		−100 (0/8)		
3c	$C_{21}H_{26}N_3O_6Cl$	451.90	231–235	83	20°, 7 days	A, $CHCl_3$	235 (9800), 262 (11 400)	0.20	0.40	1.75		20 (0/8)		
(b) Unsaturated														
3′,5′-Dihexadienoyl	$C_{21}H_{24}N_3O_6Cl$	449.90	>300	12	37°, 48 h	C, MeOH	234 (21 400), 263 (50 700)	0.10	5.80	>10	1 (0/8)	−100 (0/8)		
3′,5′-Diundec-10-enoyl	$C_{31}H_{48}N_3O_6Cl$	594.20	199–200	23	37°, 16 h	A, MeOH	234 (10 100), 263 (11 100)	0.10	0.44	0.78	24 (0/8)	−74 (0/8)		
3′,5′-Dioleoyl	$C_{45}H_{76}N_3O_6Cl$	790.59	171–172	49	37°, 16 h	A, EtOH	235 (10 500), 264 (11 300)	0.30	3.2	8.5	>141 (2/8)	>257 (8/8)	−23 (0/8)	−31 (0/9)
3′,5′-Dielaidoyl	$C_{45}H_{76}N_3O_6Cl$	790.59	177–178	43	37°, 16 h	A, MeOH	234 (10 500), 264 (11 300)	1.4	4.7	9.3	114 (0/8)	>258 (4/8)	>257 (8/8)	11 (0/8)
3′,5′-Dierucoyl	$C_{53}H_{92}N_3O_6Cl$	902.80	155–156	57	55°, 16 h	A, C, MeOH	233 (10 000), 263 (11 000)	4.0	>30	>30	>202 (2/6)	>267 (6/8)	>257 (8/8)	>257 (8/8)

[a] See ref 5. [b] Purification A, precipitation with ether followed by crystallization from the indicated solvent. Purification B, precipitation with ether followed by mild acidic hydrolysis and crystallization.[5] Purification C, precipitation with ether followed by chromatography on silicic acid using 10–20% methanol in chloroform and crystallization from the indicated solvent.

to be somewhat more soluble than their saturated counterparts and it may be noted that this is reflected in the considerably greater antiviral activities of the dioleoyl (cis-Δ^9-C_{18}) and dielaidoyl (trans-Δ^9-C_{18}) esters relative to their saturated distearoyl counterpart. As yet we have not confirmed the solubility–activity relationship by examination of solubilized preparations as was done with the 3'-monoesters.[1a]

The activities of the various diesters against L1210 leukemia in mice were examined using a single intraperitoneal injection of a solution or suspension of the drug in a standardized medium administered 24 h after standardized tumor-cell implantation.[1a] The results obtained using a standardized dose of 200 mg/kg are shown in Table I, and in certain cases the effects of other doses are also reported. It can be seen that with this dose schedule and mode of administration the short-chain diesters (C_2–C_8) show only very modest activities at the lower doses, rather similar to cycloC itself. Unlike cycloC, however, toxicity is noted with, e.g., the dibutyrate at doses as high as 1000 mg/kg. The dinonanoyl (7, R = C_8H_{17}) and didecanoyl (7, R = C_9H_{19}) esters show significant toxicity at 200 mg/kg although the latter compound is still modestly active at 50 mg/kg. The addition of even a single carbon atom to each acyl group dramatically reverses this toxicity and the intermediate-length saturated diesters (C_{11}–C_{16}) show high activity, with many long-term survivors at 200 mg/kg, while toxicity is only apparent at considerably higher doses. The 3',5'-di-*O*-dodecanoyl derivative 7 (R = $C_{11}H_{23}$), for example, completely protected the entire test group for the duration of the 30-day study. Still further increases in the length of the saturated diesters (C_{18}–C_{22}) led to a decrease in activity at lower doses and a decrease in toxicity at high doses.

The unsaturated diesters behaved in a somewhat similar way, several of the shorter chain compounds (3',5'-di-*O*-hexa-2,4-dienoyl and 3',5'-di-*O*-undec-10-enoyl cycloC) showing marked toxicity at 200 mg/kg but very little toxic or therapeutic activity at 50 mg/kg. The long-chain unsaturated diesters (oleoyl, elaidoyl, erucoyl), however, were highly active and showed decreasing toxicities. 3',5'-Di-*O*-erucoyl cycloC [7, R = *cis*-$(CH_2)_{11}CH{=}CH(CH_2)_7CH_3$], in particular, showed very high activity over the dose range 50–1000 mg/kg with no indication of toxicity at those levels.

It is interesting to note that a quite extensive series of 3',5'-diesters of 5-fluorodeoxyuridine has been prepared and examined for activity against adenocarcinoma-775 in mice by Nishizawa et al.[12] These compounds were, however, administered orally and under those conditions the rather short-chain diesters (C_4–C_8) appeared to show maximal activities while the larger esters gave indications of toxicity. The present series of compounds showed only minimal activities via the oral route, and details of the results using other modes of administration will be presented elsewhere. A considerable number of 5'-esters of araC have also been examined for activity against L1210 leukemia and viruses.[13] Some positive correlations can be drawn between the antitumor activity of the compounds, their solubility in water, and their rate of hydrolysis by plasma esterases.[13b] These results are best considered together with our own studies on 3'-esters and 3',5'-diesters of araC.[7]

The work presented in this paper provides a facile route for the synthesis of a wide range of 3',5'-diesters of cycloC. Taken together with the comparable range of 3'-esters of cycloC described in the previous paper in this series,[1a] these compounds become the starting materials for a simple synthesis of 3'-*O*-acyl and 3',5'-di-*O*-acyl araC's to be described separately.[7] Details on the further biological activities of the present compounds and on the use of other dosage regimens and modes of administration will be reported on at a later date.

Experimental Section

General Methods. The general synthetic and analytical methods used, and the protocols followed during biological studies, have been summarized in the previous paper.[1a]

General Procedures for the Preparation of 2,2'-Anhydro-1-(3',5'-di-*O*-acyl-β-D-arabinofuranosyl)cytosine Hydrochlorides (7). (a) 2,2'-Anhydro-1-(3',5'-di-*O*-decanoyl-β-D-arabinofuranosyl)cytosine Hydrochloride (7, R = C_9H_{19}). Decanoyl chloride (30.5 g, 159 mmol) was added to a stirred suspension of **6** (10.0 g, 38.3 mmol)[5] in dimethylacetamide (200 ml) and maintained at 37°. After 5 h a clear solution resulted and after 16 h TLC of an ether precipitated aliquot (1-butanol–acetic acid–water, 5:2:3) showed the reaction to be complete. The cooled solution was then added to ether (1.2 l.) giving a white precipitate that was collected, washed with ether, and dried in vacuo (26.8 g). This material was crystallized from 2-propanol giving 15.12 g (69%) of pure **7** (R = C_9H_{19}) with mp 206–208°: NMR (Me_2SO-d_6) 0.87 (t, 6, CH_3's), 1.25 (br s, 24, CH_2's), 1.5 (m, 4, $COCH_2CH_2$), 2.1 (m, 2, $COCH_2$), 2.40 (t, 2, $COCH_2$), 3.97 (dd, 1, J_{gem} = 12 Hz, $J_{4',5'a}$ = 3 Hz, $C_{5'a}H$), 4.20 (dd, 1, $J_{4',5'b}$ = 4.5 Hz, $C_{5'b}H$), 4.62 (m, 1, $C_{4'}H$), 5.41 (d, 1, $J_{3',4'}$ = 2 Hz, $C_{3'}H$), 5.73 (d, 1, $J_{1',2'}$ = 6 Hz, $C_{2'}H$), 6.64 (d, 1, $C_{1'}H$), 6.76 (d, 1, $J_{5,6}$ = 7.5 Hz, C_5H), 8.38 (d, 1, C_6H), 9.70 ppm (br s, 2, NH_2); mass spectrum (70 eV) *m/e* 533 (M^+, free base), 578, 504, 490, 476, 462, 448, 434 (degradation of acyl groups), 378 (M – $C_9H_{19}CO$), 362 (M – $C_9H_{19}CO_2$). Anal. C, H, N. See Table I for other data.

(b) 2,2'-Anhydro-1-(3',5'-di-*O*-stearoyl-β-D-arabinofuranosyl)cytosine Hydrochloride (7, R = $C_{17}H_{35}$). Stearoyl chloride (68 g, 228 mmol) was added to a stirred suspension of **6** (15.0 g, 57 mmol) in dimethylacetamide (225 ml) and stored at 40° for 48 h. At this point, TLC (1-butanol–acetic acid–water, 5:2:3) showed the reaction to be complete and the white precipitate was removed by filtration, washed with ether, and dried in vacuo. The solid was dissolved in boiling methanol (1.2 l.), filtered, and cooled giving 35.5 g (79%) of pure **7** (R = $C_{17}H_{35}$) with mp 192–193°: the compound was not sufficiently soluble to obtain a good NMR spectrum; mass spectrum (70 eV) *m/e* 757 (M^+, free base. Anal. C, H, N. See Table I for other data.

Attempts to obtain a second crop of crystalline product gave only a mixture of the 3',5'-diester and a somewhat more polar product, and further crystallization tended only to enrich this new material. A 1.0-g portion of enriched material was dissolved in hot methanol and mixed with silicic acid (10 g). The mixture was evaporated in vacuo, suspended in chloroform, and applied to the top of a column containing 75 g of silicic acid. The column was eluted with 10, 15, and 20% methanol in chloroform, which quite cleanly separated the two esters. The more polar compound was crystallized from methanol giving 280 mg of analytically pure 2,2'-anhydro-1-(3'-*O*-stearoyl-β-D-arabinofuranosyl)cytosine hydrochloride that was identical with a previously described sample[1a] by TLC, NMR, and infrared spectroscopy. The final methanolic mother liquors, after removal of all possible nucleosides, were evaporated to a small volume and added to a large excess of ether giving a white solid that was collected, washed with ether, and dried in vacuo. Recrystallization from acetonitrile–ether gave **8** as hygroscopic crystals which softened at 116° and melted at 120–123° (reported[8] mp 115–120°): NMR ($CDCl_3$) 2.61 (s, 3, Me), 3.23 ppm (s, 6, NMe_2). Anal. C, H, N.

(c) 2,2'-Anhydro-1-(3',5'-dierucoyl-β-D-arabinofuranosyl)cytosine Hydrochloride [7, R = *cis*-$(CH_2)_{11}CH{=}CH(CH_2)_7CH_3$]. Thionyl chloride (60 ml, 0.8 mol) was added to 67.7 g (0.2 mol) of erucic acid and after the initial reaction had subsided the mixture was heated to 55° for 1 h. Excess thionyl chloride was carefully removed in vacuo at 50° with stirring until no further bubbling was observed. The residue was diluted with dimethylacetamide (200 ml), **6** (13.1 g, 50 mmol) was added, and the resulting mixture was stirred at 55° for 16 h. Most of the solvent was removed under high vacuum at 70°

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.

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References and Notes

(1) (a) For paper 6 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. 119 from the Institute of Molecular Biology, Syntex Research, Palo Alto, Calif. 94304.
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(3) (a) Y. Sakai, C. Korda, M. Shimoyama, T. Kitahara, T. Sakano, and K. Kimura, *Jpn. J. Clin. Oncol.*, **2**, 57 (1972); (b) J. J. Lokich, P. L. Chawla, N. Jaffe, and E. Frei, *Cancer Chemother. Rep.*, **59**, 389 (1975).
(4) J. G. Moffatt, E. K. Hamamura, A. F. Russell, M. Prystasz, J. P. H. Verheyden, K. Yamaguchi, N. Uchida, K. Sato, S. Nishiyama, O. Shiratori, and K. Katagiri, *Cancer Chemother. Rep.*, **58**, 451 (1974).
(5) A. F. Russell, M. Prystasz, E. K. Hamamura, J. P. H. Verheyden, and J. G. Moffatt, *J. Org. Chem.*, **39**, 2182 (1974).
(6) (a) I. L. Doerr and J. J. Fox, *J. Org. Chem.*, **32**, 1462 (1967); (b) K. Kikugawa and M. Ichino, *ibid.*, **37**, 284 (1972).
(7) 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.*, following paper in this issue.
(8) See, e.g., H. H. Bosshard and H. Zollinger, *Helv. Chim. Acta*, **42**, 1659 (1959).
(9) See, e.g., (a) H. K. Hall, *J. Am. Chem. Soc.*, **78**, 2717 (1956); (b) R. R. Koganty, M. B. Shambhu, and G. A. Digenis, *Tetrahedron Lett.*, 4511 (1973).
(10) (a) S. Hanessian and N. R. Plessas, *J. Org. Chem.*, **34**, 2163 (1969); (b) D. R. Hepburn and H. R. Hudson, *Chem. Ind. (London)*, 664 (1974).
(11) R. Marumoto and M. Honjo, *Chem. Pharm. Bull.*, **22**, 128 (1974).
(12) Y. Nishizawa, J. E. Casida, S. W. Anderson, and C. Heidelberger, *Biochem. Pharmacol.*, 14, 1605 (1965).
(13) (a) D. T. Gish, R. C. Kelly, G. W. Camiener, and W. J. Wechter, *J. Med. Chem.*, 14, 1159 (1971); (b) W. J. Wechter, M. A. Johnson, C. M. Hall, D. T. Warner, A. E. Berger, A. H. Wenzel, D. T. Gish, and G. L. Neil, *J. Med. Chem.*, 18, 339 (1975).
(14) R. M. Scribner, U.S. Patent 3458505 (1969); *Chem. Abstr.*, 71, 91773 (1969). We are grateful to E. I. du Pont de Nemours and Co. for a sample of the corresponding acid.

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]