The oral dose of drug required to produce a 50 mmHg reduction in systolic blood pressure (ED_{-50SBP}) was calculated by regression analysis. The data in Table IV are presented as ED_{-50SBP} (mg/kg, po) with 95% fiducial limits.

α_1-Adrenergic Antagonist Activity. The potency of these compounds as α_1-adrenergic antagonists was determined by using the following procedure: adult male SHR or mongrel dogs were anesthetized and bilaterally vagotomized. A carotid artery and jugular vein were cannulated for monitoring mean arterial blood pressure and drug administration, respectively. The percent inhibition of α_1-adrenergic receptor activation was quantified by measuring pressor responses to phenylephrine before and after antagonist treatment. The dose of antagonist required to produce a 50% inhibition of the phenylephrine pressor response (ED_{50}; rats) or a 20-fold rightward shift in the dose–response curve (DR_{20}; dogs) was calculated by regression analysis. The data in Table VII are presented as ED_{50} or DR_{20} values (μg/kg, iv) with 95% fiducial limits.

Acknowledgment. We thank Dr. M. L. Cotter and staff for spectral measurements and elemental analyses and Dr. David Graden for NOE experiments and useful discussions.

Registry No. **3**, 110164-48-2; **3** (free base), 110164-06-2; **4**, 110164-15-3; **5**, 110164-21-1; **12**, 110164-10-8; **13**, 110164-09-5; **14**, 110164-53-9; **14** (free base), 110187-04-7; **15**, 110164-08-4; **16**, 111308-59-9; **17**, 110164-51-7; **17** (free base), 110164-11-9; **18**, 110164-07-3; **19**, 110164-52-8; **19** (free base), 110164-12-0; **20**, 114942-96-0; **20** (free base), 114942-97-1; **21**, 110164-13-1; **22**, 114942-98-2; **23**, 110164-56-2; **23** (free base), 110164-14-2; **24**, 114942-99-3; **25**, 110164-18-6; **26**, 110164-19-7; **27**, 110164-17-5; **28**, 110164-16-4; **29**, 110164-20-0; **30**, 110164-25-5; **31**, 110164-24-4; **32**, 110164-23-3; **33**, 110164-22-2; **34**, 110164-26-6; **35**, 110164-28-8; **36**, 114943-00-9; **37**, 110164-29-9; **38**, 110164-30-2; **39**, 110164-31-3; **40**, 110164-32-4; **41**, 110164-33-5; **42**, 110164-36-8; **43**, 110164-37-9; **44**, 110164-39-1; **45**, 110164-40-4; **46**, 110164-42-6; **47**, 110164-41-5; **48**, 110164-43-7; **49**, 110164-44-8; **50**, 114943-01-0; **51**, 110164-34-6; **52**, 110187-05-8; **53**, 110197-52-9; **54**, 110187-06-9; **55**, 110164-46-0; **56**, 110164-38-0; **57**, 110164-35-7; **58**, 110164-47-1; **59**, 110164-54-0; **60**, 114943-02-1; **61**, 110164-55-1; **62**, 110164-57-3; **63**, 110164-63-1; **64**, 110164-65-3; **65**, 110164-71-1; **66**, 110164-74-4; **67**, 110164-73-3; **68**, 110164-76-6; **69**, 110164-50-6; **69** (free base), 110164-49-3; **70**, 110164-58-4; **71**, 114943-03-2; **72**, 110164-61-9; **73**, 110164-62-0; **74**, 110164-60-8; **75**, 110164-59-5; **76**, 110164-64-2; **77**, 110164-66-4; **78**, 110164-70-0; **79**, 110164-69-7; **80**, 114943-04-3; **81**, 110164-67-5; **82**, 110164-72-2; **83**, 110164-75-5; **84**, 110164-77-7; $BrCH_2CH{=}CH_2$, 106-95-6; $BrCH_2C{\equiv}CH$, 106-96-7; $Br(CH_2)_7CH_3$, 111-83-1; $Br(CH_2)_2CO_2Me$, 3395-91-3; $Br(CH_2)_3CO_2Et$, 2969-81-5; $Br(CH_2)_3CH_3$, 109-65-9; $Br(CH_2)_4CO_2Et$, 14660-52-7; $ClCOC(CH_3)_3$, 3282-30-2; $ClCO(CH_2)_4CH_3$, 142-61-0; ClCOPh, 98-88-4; p-$ClCOC_6H_4Cl$, 122-01-0; p-$ClCOC_6H_4OMe$, 100-07-2; $ClCO_2Et$, 541-41-3; methyl 4-aminothiophene-3-carboxylate, 69363-85-5; methyl 4-amino-2-methylthiophene-3-carboxylate, 114943-05-4; methyl 3-aminothiophene-2-carboxylate, 22288-78-4; ethyl 2-aminothiophene-3-carboxylate, 31891-06-2; ethyl 2-amino-4-methylthiophene-3-carboxylate, 43088-42-2; ethyl 2-amino-4,5-dimethylthiophene-3-carboxylate, 4815-24-1; 2-chloroethyl isocyanate, 1943-83-5; 3-chloropropyl isocyanate, 13010-19-0; 4-(2-methoxyphenyl)piperazine hydrochloride, 5464-78-8; 4-(3-methoxyphenyl)piperazine hydrochloride, 16015-70-6; 4-(4-methoxyphenyl)piperazine hydrochloride, 84145-43-7; 4-phenylpiperazine hydrochloride, 2210-93-7; 4-(2-chlorophenyl)piperazine hydrochloride, 41202-32-8; 4-(2-tolyl)piperazine hydrochloride, 95356-15-3; 4-(3-chlorophenyl)piperazine hydrochloride, 13078-15-4; 4-(4-chlorophenyl)piperazine hydrochloride, 13078-12-1; 4-(4-fluorophenyl)piperazine hydrochloride, 16141-90-5; 4-(2-ethoxyphenyl)piperazine hydrochloride, 83081-75-8; isobutyryl chloride, 79-30-1.

Nucleoside Conjugates. 10. Synthesis and Antitumor Activity of 1-β-D-Arabinofuranosylcytosine 5′-Diphosphate–1,2-Dipalmitins[1]

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Three 1-β-D-arabinofuranosylcytosine 5′-diphosphate–1,2-dipalmitins from L-, D-, and DL-α-dipalmitoylphosphatidic acids have been synthesized and their antitumor activity against two ara-C^2 resistant L1210 lymphoid leukemia sublines in mice were evaluated. These new prodrugs of ara-C include ara-CDP-L-dipalmitin (**1**), ara-CDP-D-dipalmitin (**2**), and ara-CDP-DL-dipalmitin (**3**). The L and DL isomers produced significant increase in life span (>400%) and four to five long-term survivors (>45 days) out of six animals bearing ip implanted partially ara-C resistant L1210 subline [L1210/ara-C (I)], while the D isomer displayed a marginal activity (ILS 100–121%). In contrast, the L isomer was completely ineffective against deoxycytidine kinase deficient ara-C resistant L1210 subline [L1210/ara-C (II)]. However, the results demonstrate that the L and DL isomers of ara-CDP-dipalmitin are promising new prodrugs of ara-C with improved efficacy.

1-β-D-Arabinofuranosylcytosine 5′-diphosphate–L-1,2-dipalmitin (ara-CDP-L-dipalmitin, **1**)[2] (Figure 1) is an ara-C conjugate of phospholipid that has demonstrated a superior antitumor activity over ara-C independent of the treatment schedules.[3-6] The DL racemic mixture, ara-CDP-DL-diplamitin (**3**), has also shown promising therapeutic results.[7-9] To determine stereochemical significance

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(2) The abbreviations used are: ara-C, 1-β-D-arabinofuranosylcytosine; ara-CMP, 1-β-D-arabinofuranosylcytosine 5′-monophosphate; ara-CDP-L-(D or DL)-dipalmitin; 1-β-D-arabinofuranosylcytosine 5′-diphosphate-L-(D or DL)-1,2-dipalmitin; ribo-CDP-L-dipalmitin, cytidine 5′-diphosphate-L-1,2-dipalmitin; ara-CMP-morpholidate, 1-β-D-arabinofuranosylcytosine 5′-monophosphoromorpholidate 4-morpholine-*N*,*N*′-dicyclohexylcarboxamidinium salt.

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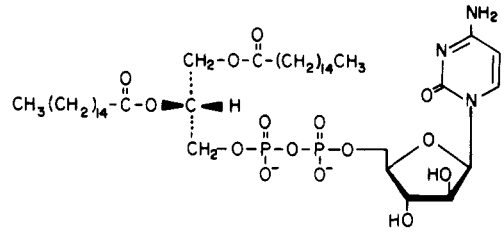
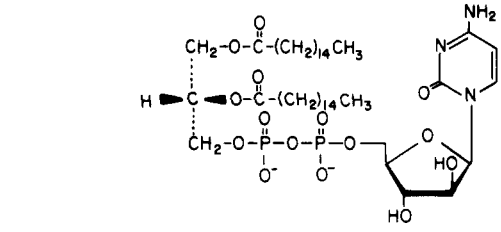
Table I. Ara-CDP-dipalmitins

compd	mp, °C	yield, %	$[\alpha]^{22}_D$,[a] deg	formula[b]	UV_{max}, nm ($\epsilon \times 10^{-3}$) neutral[c]	acid[d]	base[e]	^1H NMR data[f] 2-CH
1	195–198[g]	42	+40.0 (c 0.31)	$C_{44}H_{79}N_3O_{15}P_2 \cdot 2Na \cdot H_2O$[h]	273 (8.82)	283 (12.6)	273 (9.03)	5.26 (m)
2	195–198	39	+25.1 (c 0.39)	$C_{44}H_{79}N_3O_{15}P_2 \cdot 2Na \cdot 6CH_3OH$	273 (9.05)	283 (13.4)	273 (10.2)	5.19 (m)
3	197–199	48	+25.8 (c 0.24)	$C_{44}H_{79}N_3O_{15}P_2 \cdot 2Na \cdot 0.5H_2O$[i]	273 (9.68)	282 (14.1)	273 (9.93)	5.23 (m)

[a] Solvent: $CHCl_3$–MeOH–H_2O (2:3:1). [b] Analyses for C, H, N, and P for all compounds. [c] $CHCl_3$–MeOH–H_2O (2:3:1). [d] $CHCl_3$–MeOH–0.6 N HCl (2:3:1). [e] $CHCl_3$–MeOH–0.6 N NaOH (2:3:1). [f] All shifts of the *sn*-2-methine proton measured in δ from Me_4Si. [g] Literature[3] mp 185–189 °C. [h] N: calcd, 4.14; found, 3.56. [i] N: calcd, 4.17; found, 3.55.





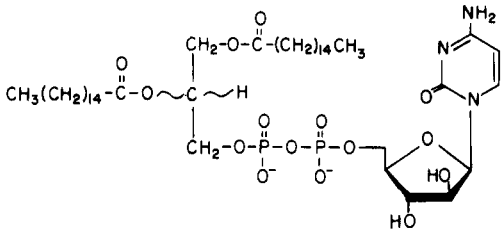


Figure 1. Structures of ara-CDP-dipalmitins.

of the 1,2-dipalmitin moiety, we have now synthesized the D isomer, ara-CDP-D-dipalmitin (**2**), and also improved the synthetic procedures of the L isomer (**1**) and the DL racemic mixture (**3**).

This paper describes the scale-up synthetic procedures of these ara-CDP-dipalmitins (**1–3**) and their antitumor effects against L1210 lymphoid leukemia in mice.

Chemistry

A major problem for synthesis of conjugates **1–3** has been the availability of pure phosphatidic acids **7–9** (Figure 2). Previously, L-α-dipalmitoylphosphatidic acid (**7**) was prepared by enzymatic hydrolysis of L-α-dipalmitoylphosphatidylcholine with phospholipase D[5] or purchased commercially. This was not suitable for the scale-up synthesis. Thus, we improved the chemical procedures for the preparation of dipalmitins **4–6**[10–12] and phosphatidic

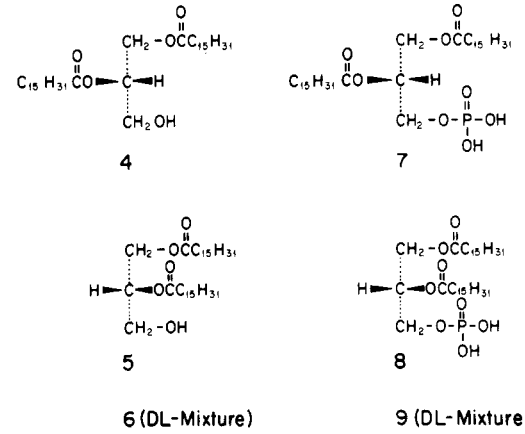


Figure 2. Structures of dipalmitins and α-dipalmitoylphosphatidic acids.

acids **7–9**.[8,13] 1,2-*O*-Isopropylidene-*sn*-glycerol was prepared from D-mannitol according to the method published previously.[14] This was then reacted with benzyl chloride in the presence of $NaNH_2$, and the isopropylidene group of 1,2-isopropylidene-3-*O*-benzyl-*sn*-glycerol was removed by refluxing in aqueous AcOH. The resulting 3-*O*-benzyl-*sn*-glycerol was acylated with palmitoyl chloride in the presence of pyridine and 1,2-*O*-dipalmitoyl-3-*O*-benzyl-*sn*-glycerol was purified by crystallization from a large amount of boiling 95% EtOH. The benzyl group of this compound was removed by catalytic hydrogenolysis with 10% Pd/C, and L-dipalmitin (**4**) was obtained in good yield (51% based on 1,2-*O*-isopropylidene-*sn*-glycerol). Compound **4** was phosphorylated with $POCl_3$ and Et_3N at 0.5 °C as reported earlier[15] with some modification of solvent and purification.[16] Compound **7** was purified by successive crystallization from hexanes and Et_2O, and the final yield was 76%. The DL-phosphatidic acid (**9**) was prepared in an analogous manner from 1,2-*O*-isopropylidene-*rac*-glycerol. The D isomer (**8**) was prepared by the following procedure. 1,2-*O*-Isopropylidene-*sn*-glycerol was first acylated with palmitoyl chloride, and the isopropylidene group was removed with 80% HOAc at 90 °C. 3-*O*-Palmitoyl-*sn*-glycerol was tritylated with trityl chloride and pyridine, and the resulting 1-*O*-trityl-3-*O*-palmitoyl-*sn*-glycerol was acylated with palmitoyl chloride in the presence of pyridine. 1-*O*-Trityl-2,3-*O*-dipalmitoyl-*sn*-glycerol was then detritylated by a column of 10% boric acid impregnated silica gel to give D-dipalmitin (**5**) in 72% yield. Compound **5** was then phospho-

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Table II. Biochemical Properties of L1210 Lymphoid Leukemia Sublines

cell line	ara-C uptake rate ± SD, pmol of ara-C/min per 10^6 viable cells	deoxycytidine kinase activity, pmol of phosphorylated/min per mg of protein
L1210/0	3.78 ± 1.07 (7)[a]	33.43 ± 8.19 (3)
L1210/ara-C (I)	2.47 ± 0.52 (3)	37.80 ± 10.67 (3)
L1210/ara-C (II)	0.105 ± 0.018 (3)	0.82 ± 0.30 (5)

[a] Number of determinations performed is given in parentheses.

rylated with $POCl_3$ and Et_3N at 0–5 °C to give the D-phosphatidic acid (**8**).

Structural assignment of dipalmitins **4–6** and phosphatidic acids **7–9** was confirmed by ^1H NMR spectrometry and optical rotation. The *sn*-2-methine proton of the 1,2-dipalmitins (**4–6**) and their 3-phosphates (**7–9**) gave a first-order quintet at 5.09–5.23 ppm, whereas it was a second-order multiplet at 3.83–3.88 ppm in 3-*O*-benzyl-*sn*-glycerol and 3-*O*-palmitoyl-*sn*-glycerol. These values are in good agreement with those for isomeric lysophosphatidylcholines reported previously.[17] Condensation of **7–9** with ara-CMP-morpholidate[3] in pyridine gave conjugates **1–3** in an overall yield of 40–50%, and their structures were verified by elemental analysis and ^1H NMR and UV spectrometry (Table I).

Biological Results

Three ara-CDP-dipalmitins (**1–3**) were evaluated for in vivo antitumor activity against three L1210 leukemia cell lines listed in Table II. Antitumor results against L1210/0 have been reported previously.[6] Antitumor effects against ip implanted L1210/ara-C (I) and L1210/ara-C (II) lymphoid leukemia in DBA/2J mice are shown in Table III. L1210/ara-C (I) was found to be partially resistant to ara-C, when ara-C uptake and antitumor results were observed, while L1210/ara-C (II) was completely resistant to ara-C. L1210/-ara-C (I) produced a similar deoxycytidine kinase activity to L1210/0. However, the ara-C uptake rate was lower than that of L1210/0 (Table II).

In Table III, the untreated mice died on days 8–11 after L1210/ara-C (I) cell implantation. Under a treatment regimen of daily ip injections of our optimum dose (60 mg, 247 μmol/kg per day) of ara-C for 5, 9, and 15 days, the maximal ILS values obtained were 65, 82, and 147%, respectively. In contrast, ara-CDP-L-dipalmitin (**1**), administered ip as a single dose, in three doses over a 9-day period, or in five doses over a 5-day period, produced ILS values of >374 to >400% with four to five 45-day survivors out of six animals. The DL mixture (**3**) also produced ILS values of 233 to >400% with two to four 45-day survivors with the three treatment schedules. However, the D isomer (**2**) showed less activity (ILS, 100–121%) than the L and the DL isomer. Mixtures of ara-CMP and the phosphatidic acid (**7** or **8**) at the same molar doses as the corresponding conjugates produced ILS values of 5–16%. The lipophilic prodrugs, 5′-*O*-palmitoyl-ara-C[18] and N^4-palmitoyl-ara-C[19] were also effective (ILS 139–144%).

Table III also shows effects of ara-C, ara-CDP-L-dipalmitin (**1**), and the lipophilic prodrugs on L1210/ara-C (II). These results indicated that, like ara-C, ara-CDP-L-dipalmitin and the lipophilic prodrugs seemed to require deoxycytidine kinase to be effective. Further biochemical pharmacology of ara-CDP-L-dipalmitin is under investigation.

Discussion

Results obtained in this study and the previous works with ara-CDP-dipalmitins (**1–3**)[6] and other lipophilic ara-C conjugates[20] have demonstrated that the conjugates of this type require that the possible carrier moiety is not only lipophilic but also naturally occurring. In fact, ara-CDP-L-dipalmitin is the 2′ epimer of ribo-CDP-L-dipalmitin, which is one of naturally occurring ribo-CDP-L-diacylglycerols containing mostly different fatty acyl chains esterified to respective *sn*-1 and *sn*-2 positions. The ribo-CDP-L-diacylglycerols are the immediate biosynthetic precursors to phosphatidylinositol,[21,22] which is now recognized as the source of second messengers in receptor-mediated Ca^{2+} mobilization[23,24] and protein kinase C activation.[25,26] Thus, the conjugate retains its affinity to the serum protein, cell surface, and plasma membrane, and it would be hydrolyzed enzymatically to give ara-CMP and phosphatidylinositol or phospholipid.[7] However, lack of the activity against deoxycytidine kinase deficient L1210/ara-C (II) suggests that ara-CDP-L-dipalmitin may be hydrolyzed enzymatically in the membrane,[27] and the resulting ara-CMP is converted back to ara-C by membrane-bound 5′-nucleotidase and other phosphatases. Further work on the biochemical study of the conjugate will be necessary to confirm this. Some favorable properties found to ara-CDP-L-dipalmitin are (1) superior antitumor activity, independent of the treatment schedule, sustained release form, (2) resistance to hydrolysis by cytidine deaminase,[4] (3) rapid interaction with serum lipoprotein,[28] which would have a possible role in the transport of the conjugate, and (4) solution by sonication. These results along with previous data,[4-6,9,16] demonstrate that the ara-C conjugates of phospholipids have the potential interest in cancer chemotherapy.

Experimental Section

Synthesis. Melting points were taken on a Mel-Temp capillary melting point apparatus and are uncorrected. Nuclear magnetic resonance (^1H NMR) spectra were recorded on a Varian Associate EM-390 spectrometer. The chemical shift values are expressed in δ values (ppm) relative to tetramethylsilane as an internal standard. UV absorption spectra were recorded on a Perkin-Elmer Lambda 4A spectrophotometer. The optical rotations were measured on a Perkin-Elmer 241 polarimeter. AG1-X8 (Bio-Rad), [(diethylamino)ethyl]cellulose (DE-52, Whatman), Dowex 50W-X8 (Bio-Rad), and Amberlite CG-50 (Mallinckrodt) were used for column chromatography. Evaporations were carried out on a rotary evaporator under reduced pressure applied by a tap-water aspirator or a vacuum pump with a bath temperature of under 30 °C. TLC was performed on glass plates coated with a 0.25-mm layer of silica gel PF-254 (Brinkman) and on polygram sil G/UV 254 (Brinkman) with use of the following solvent systems: (A) $CHCl_3$, (B) $CHCl_3$–MeOH (95:5), (C) $CHCl_3$–MeOH–H_2O–HOAc (25:15:4:2), and (D) *i*-PrOH–H_2O–concentrated NH_4OH (7:2:1). UV-absorbing compounds were detected by visualization under a UV lamp (254 nm), and phosphorus-containing compounds were

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detected with a modified Dittmer–Lester spray.[29] The organic compounds were also detected by charring after spraying with the above reagent. Elemental analyses were performed by Galbraith Laboratories, Knoxville, TN. When analyses are reported only by the element symbols, results are within ±0.4% of the theoretical values including given numbers of H_2O of hydrations or MeOH solvate unless noted otherwise. Presence of MeOH in compound **2** was confirmed by ^1H NMR spectroscopy.

Ara-CMP,[30] ara-CMP-morpholidate,[3] and 1,2-isopropylidene-*sn*-glycerol[14] were prepared by a literature procedure.

3-*O*-Benzyl-*sn*-glycerol. This compound was prepared by a literature procedure.[31] To a mixture of 1,2-isopropylidene-*sn*-glycerol[14] (132 g, 1 mol) and benzene (250 mL) was added $NaNH_2$ (39 g, 1 mol) in small portions, and the mixture was refluxed for 1 h. Benzyl chloride (200 g, 1.2 mol) was added dropwise to the hot mxture within 1 h, and boiling was continued for 20 h. After being cooled to room temperature, the mixture was extracted with 200 mL of H_2O, and the organic layer was evaporated at 30 °C in vacuo followed by distillation at 85–87 °C (0.5 mmHg), which yielded 167 g (68%) of 1,2-isopropylidene-3-*O*-benzyl-*sn*-glycerol; $[\alpha]^{22}_D$ +20.45° (neat). This was dissolved in 220 mL of *i*-PrOH, 20 mL of HOAc, and 100 mL of H_2O, and the solution was refluxed for 24 h. The solvent was evaporated in vacuo, and then the residue was distilled at 117–122 °C (0.5 mmHg): yield of product, 106 g (91%); $[\alpha]^{22}_D$ +7.25° (neat) (lit.[32] $[\alpha]^{20}_D$ +5.8°); ^1H NMR ($CDCl_3$ δ 3.40 (2, t, 1-CH_2, J = 5 Hz), 3.50 (2 m, 3-CH_2), 3.83 (1, quintet, J = 5 Hz, 2 CH), 4.42 (2, s, C$H_2C_6H_5$), 7.20 (5, s, C_6H_5).

1,2-*O*-Dipalmitoyl-*sn*-glycerol (L-Dipalmitin, 4). To a solution of 3-*O*-benzyl-*sn*-glycerol (18.2 g, 0.1 mol), pyridine (16.75 g, 0.212 mol), and 100 mL of benzene was added dropwise 55 g (0.2 mmol) of palmitoyl chloride for a period of 30 min at room temperature. The mixture was heated at 60–70 °C for 1 day. After being cooled to room temperature, the mixture was mixed with Et_2O (600 mL), and the organic phase was washed with H_2O, 0.2 N H_2SO_4, saturated $NaHCO_3$, and H_2O (200 mL each). The organic layer was dried over Na_2SO_4 and evaporated. The residue was crystallized from large volume of boiling 95% ethanol, which gave 55.3 g (84%) of 1,2-*O*-dipalmitoyl-3-*O*-benzyl-*sn*-glycerol: mp 42.0–42.5 °C; $[\alpha]^{22}_D$ +6.0° (*c* 8.5, $CHCl_3$). This compound (20 g) was hydrogenated in the presence of 1 g of 10% Pd/C in 250 mL of *n*-hexane at 20–50 psi for 1 day. The mixture was warmed in a water bath, and the catalyst was removed by filtration. The product (**4**) was crystallized from *n*-hexane: yield 16.8 g (97%); mp 66–68 °C (lit.[10] mp 67–67.5 °C); $[\alpha]^{22}_D$ −2.21° (*c* 9.9, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 0.88 (6, t, J = 6 Hz, 2-CH_3), 1.22–1.73 (52 m, 2 (CH_2)$_{13}$), 2.33 (4, m, 2 CH_2CO), 3.70 (2, t, J = 5 Hz, 3-CH_2), 4.25 (2, d, J = 5 Hz, 1-CH_2), 5.09 (1, quintet, J = 5 Hz, 2-CH). *rac*-1,2-*O*-Dipalmitoylglycerol (DL-dipalmitin, **6**) was prepared in an analogous manner from *rac*-3-*O*-benzylglycerol: yield 72%; mp 66–68 °C.

L-α-Dipalmitoylphosphatidic Acid (7). To a mixture of $POCl_3$ (2.25 g, 15 mol), Et_3N (1.5 g, 15 mmol), and *n*-hexane (15 mL) was added dropwise **4** (5.69, 10 mmol) in toluene (40 mL) at 0–5 °C for 30 min, and then the mixture was stirred at room temperature overnight. Water (5 mL) was added to the mixture, and the suspension was stirred at room temperature for 1 h. The mixture was then partitioned between 300 mL of Et_2O and 200 mL of H_2O. The organic layer was washed with H_2O (2 × 50 mL) and dried over Na_2SO_4. After being evaporated to dryness, the residue was crystallized from *n*-hexane: yield 4.9 g (76%), mp 59–60 °C (lit.[13] 62.5–63.5 °C); $[\alpha]^{22}_D$ +4.0° (*c*, 10,0 $CHCl_3$).

DL-α-Dipalmitoylphosphatidic acid (**9**) was prepared in an analogous manner from **6**: yield 77%; mp 59–60 °C (lit.[8] mp 59–62 °C); ^1H NMR ($CDCl_3$) δ 0.90 (6, t, J = 6 Hz, 2 CH_3), 1.18–1.79 (52, m, 2 (CH_2)$_{13}$), 2.37 (4, m, 2 CH_2CO), 4.26 (4, m, 1-CH_2, 3-CH_2), 5.23 (1, quintet, J = 5 Hz, 2-CH).

D-α-Dipalmitoylphosphatidic acid (**8**) was also prepared in an analogous manner from **5**: yield 75% mp 60–62 °C; $[\alpha]^{22}_D$ +0.1° (*c* 5.0, $CHCl_3$). Anal. ($C_{35}H_{69}O_8P$) C, H, P.

3-*O*-Palmitoyl-*sn*-glycerol. To an ice-cold solution of 1,2-*O*-isopropylidene-*sn*-glycerol (4 g, 0.03 mol) in toluene (50 mL) and pyridine (3.1 mL) was added dropwise palmitoyl chloride (9.62 g, 0.035 mol) in toluene (50 mL) for a period of 30 min. The reaction mixture was stirred 2 h at 0 °C and then stirred at room temperature for 16 h. The mixture was filtered, and the filtrate was washed with 5% H_2SO_4, saturated $NaHCO_3$, and H_2O (100 mL each). After being dried over Na_2SO_4, the organic layer was evaporated to dryness to give 1,2-*O*-isopropylidene-3-*O*-palmitoyl-*sn*-glycerol in a quantitative yield. Without further purification, the white residue was mixed with 100 mL of 80% HOAc and heated at 90 °C for 2 h and then stirred at room temperature for 16 h. The reaction mixture was evaporated to dryness, and the residue was triturated with MeOH (15 mL). The crude product was recrystallized from Me_2CO to give 7.4 g (yield 75%): mp 62–64 °C; ^1H NMR ($CDCl_3$) δ 0.87 (3, t, J = 6 Hz, CH_3), 1.21–1.60 (26, m, (CH_2)$_{13}$), 2.32 (2, t, J = 7 Hz, CH_2CO), 3.60 (2, dd, J = 5.5 Hz, 3-CH_2), 3.88 (1, quintet, J = 5.5 Hz, 1-CH_2).

1-*O*-Trityl-3-*O*-palmitoyl-*sn*-glycerol. A mixture of 3-*O*-palmitoyl-*sn*-glycerol (4.2 g, 13 mmol) and trityl chloride (4.1 g, 14.9 mmol) in 40 mL of anhydrous pyridine was stirred at 50 °C overnight. The reaction mixture was then poured into ice–water (500 mL), and the resulting precipitate was collected on a filter followed by drying in vacuo. The crude product was recrystallized from $CHCl_3$ and Et_2O: yield 6.8 g (91%); mp 93–95 °C.

1-*O*-Trityl-2,3-*O*-dipalmitoyl-*sn*-glycerol. To an ice-cold solution of 1-*O*-trityl-3-*O*-palmitoyl-*sn*-glycerol (7.1 g, 12 mmol) and pyridine (1.32 mL) in toluene (50 mL) was added dropwise a solution of palmitoyl chloride (3.85 g, 14 mmol) in toluene (30 mL), and then the mixture was stirred at room temperature overnight. The precipitate was removed by filtration, and the filtrate was evaporated to a syrup. The residue was dissolved in Et_2O (80 mL), and the solution was washed with 5% H_2SO_4 (50 mL), saturated $NaHCO_3$ (2 × 5 mL), and H_2O (3 × 50 mL). This was dried over Na_2SO_4 and evaporated to dryness, which gave a quantitative yield: ^1H NMR ($CDCl_3$) δ 0.82 (6, m, 2 CH_3), 1.26–1.58 (52, m, 2 (CH_2)$_{13}$), 2.25 (4, m, 2 CH_2CO), 3.20 (2, d, J = 5 Hz, 3-CH_2), 4.22 (2, d, J = 5 Hz, 1-CH_2), 5.16 (1, quintet, J = 5 Hz, 2-CH), 7.12–7.38 (15, m, (C_6H_5)$_3$).

2,3-*O*-Dipalmitoyl-*sn*-glycerol (D-Dipalmitin, 5). A solution of 1-*O*-trityl-2,3-*O*-dipalmitoyl-*sn*-glycerol (9.7 g, 12 mmol) in 50 mL of petroluem ether (bp 30–60 °C) was applied to a column (2.5 × 40 cm) of 10% boric acid impregnated silica gel (100–200 mesh).[32] The column was eluted with petroleum ether (100 mL), petroleum ether–Et_2O (95:5) (650 mL), and petroleum ether–Et_2O (3:1) (2250 mL). The latter eluate (2250 mL) was evaporated to dryness and the residue was crystallized from 95% EtOH: yield 4.92 g (72%); mp 66–68 °C; $[\alpha]^{22}_D$ +1.4° (*c* 2.3, $CHCl_3$).

Ara-CDP-1,2-*O*-dipalmitoyl-*sn*-glycerol (Ara-CDP-L-dipalmitin, 1). An anhydrous mixture of **7** (6.64 g, 10.23 mmol) and ara-CMP morpholidate[3] (5.33 g, 7.77 mmol) in 600 mL of anhydrous pyridine was stirred at room temperature for 6–10 days. The mixture was then evaporated to dryness, and the residual pyridine was removed by evaporation with toluene (20 mL). The residue was dissolved in 1000 mL of $CHCl_3$–MeOH–H_2O (2:3:1), and 100 mL of HOAc was added followed by stirring at room temperature for 30 min. The mixture was diluted with 150 mL of $CHCl_3$, and the $CHCl_3$ layer was separated out. The aqueous layer was extracted with $CHCl_3$ (2 × 100 mL), and the combined $CHCl_3$ solution was evaporated to dryness. The residue was dissolved in 500 mL of $CHCl_3$–MeOH–H_2O (2:3:1) (filter if necessary), and the clear solution was applied to a DE-52 (AcO^-) column (2.5 × 50 cm, jacked, 5 °C) equilibrated with the same solvent. The column was first washed with the solvent (1000 mL) and then eluted with a linear gradient of 0–0.15 M NH_4OAc in $CHCl_3$–MeOH–H_2O (2:3:1) (1.5 L each). The fractions (1200–2000 mL) containing the product as NH_4 salt were evaporated to a small volume (30 mL). The solid was filtered and washed with H_2O and Me_2CO. This was dissolved in $CHCl_3$–MeOH–H_2O (2:3:1) (1 L), and the solution was passed through a CG-50 (Na^+) column (2.5 × 8.0 cm). The column was eluted further with the same solvent, and the eluate containing the product was evaporated to dryness. The residue was recrystallized from $CHCl_3$–Me_2CO. The product was dried in vacuo over P_2O_5: yield 3.23 g (42%); mp 195–198 °C; ^1H NMR ($CDCl_3$–CD_3OD–D_2O, 2:3:1) δ 0.90 (6, t, J = 7 Hz, 2 CH_3), 1.30–1.60 (52, m, 2 (CH_2)$_{13}$), 2.32 (4, m, 2

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Table III. Antitumor Activity against Ip Implanted L1210/ara-C Lymphoid Leukemia in Mice[a]

cell line	compound	treatment schedule, qd	active dose range,[b] mg (μmol)/kg per day	optimum dose,[c] mg (μmol)/kg per day	wt change[d] (g/mouse) on day 8	survival days			45-day survivors
						range	median (T/C)[e]	%ILS[f]	
L1210/ara-C (I)	ara-C	1		200 (822)	+3.60	9–12	10.5/9.5	17	0
		1, 5, 9	100 (411)–133 (548)	100 (411)	+2.60	12–13	12.0/8.0	50	0
		1–5	10(41)–100 (411)	60 (247)	−0.87	13–15	14.0/8.5	65	0
		1–9		60 (247)	−1.80	15–18	15.5/8.5	82	0
		1–15		60 (247)	−0.93	17–21	21.0/8.5	147	0
	ara-CDP-L-dipalmitin (1)	1	200 (195)–400 (391)	300 (293)	−0.04	8–>45	>45.0/9.0	>400[g]	4
		1, 5, 9	100 (98)–167 (163)	133 (130)	−0.53	35–>45	>45.0/9.5	>374[g]	5
		1–5	20 (20)–60 (59)	60 (59)	−2.72	29–>45	>45.0/9.0	>400[g]	5
	ara-CMP + 7[h]	1		95 and 190 (293 each)	−0.50	9–11	10.0/9.5	5	0
		1–5		19 and 38 (59 each)	−1.08	7–14	11.0/9.5	16	0
	ara-CDP-D-dipalmitin (2)	1	200 (195)–400 (391)	400 (391)	+0.18	17–35	19.0/9.0	100	0
		1–5	40 (39)–80 (78)	80 (78)	−0.48	17–29	21.0/9.5	121	0
	ara-CMP + 8[h]	1		95 and 190 (293 each)	−0.82	10	10.0/9.5	5	0
		1–5		19 and 38 (59 each)	−1.58	11–12	11.0/9.5	16	0
	ara-CDP-DL-dipalmitin (3)	1	200 (195)–400 (391)	400 (391)	−2.07	32–>45	>45.0/9.0	>400[g]	4
		1, 5, 9	67 (65)–133 (130)	100 (98)	−1.35	14–>45	37.5/9.0	317	2
		1–5	40 (39)–80 (78)	60 (59)	−2.92	15–>45	30.0/9.0	233	2
	5′-*O*-palmitoyl-ara-C	1	50 (104)–300 (623)	100 (208)	−2.90	8–>45	21.5/9.0	139	2
	N^4-palmitoyl-ara-C	1	50 (104)–300 (623)	300 (623)	−1.20	16–>45	22.0/9.0	144	1
L1210/ara-C (II)	ara-C	1–5		100 (411)		7–8	7.0/9.0	−22	0
				200 (822)		7	7.0/9.0	−22	0
	ara-CDP-L-dipalmitin (1)	1		300 (293)		7–8	8.0/9.0	−11	0
		1–5		60 (59)	−0.30	8–9	8.0/9.0	−11	0
	5′-*O*-palmitoyl-ara-C	1		100 (208)	−1.90	7–9	7.5/9.0	−17	0
	N^4-palmitoyl-ara-C	1		300 (623)	−4.30	8–9	8.0/9.0	−11	0

[a] Each group of six DBA/2J mice (wt 20–30 g) received ip inoculation of 1×10^5 cells on day 0. Treatment (ip) were initiated 24 h after tumor inoculation. Animals were observed daily until death or 45 days. [b] Dose producing an increase in life span ≥25% over the controls. [c] Dose producing greatest increase in life span. [d] Weight change for the control studies (24 mice)e averaged +1.14 ± 2.55 (SD) g/mouse. [e] Calculated based on survivors according to the NCI protocols.[35] Median survival day of 24 control animals was 9.13 ± 0.25 (SD) days. [f] Increase in life span: (T/C − 1) × 100. [g] As of day 45 (final day of observation). [h] Mixture of ara-CMP and 7 or 8.

CH_2CO), 4.00–4.40 (9, m, *H*-2′, *H*-3′, *H*-4′, *H*-5′, 1-CH_2, 3-CH_2), 5.26 (1, m, 2-C*H*), 6.06 (1, d, J = 7 Hz, cytosine *H*-5), 6.18 (1, d, J = 5 Hz, *H*-1′), 7.96 (1, d, J = 7 Hz, cytosine *H*-6).

ara-CDP-2,3-*O*-dipalmitoyl-*sn*-glycerol (**2**) and ara-CDP-*rac*-1,2-*O*-dipalmitoylglycerol (**3**) were prepared in an analogous manner.

Biological Studies. Tumor Cells and Animals. L1210/0 and L1210/ara-C (I) lymphoid leukemia cells were purchased from Arthur D. Little, Inc. (Cambridge, MA) and L1210/ara-C (II) was obtained from Dr. Ralph J. Bernacki from Roswell Park Memorial Institute. The cells were routinely transplanted in DBA/2J mice, which were supplied by Roswell Park Memorial Institute. All operations were conducted at 4 °C unless specified otherwise.

Preparation of Cells. Ascites fluid from two animals bearing 6–8 day old L1210/0, L1210/ara-C (I), or L1210/ara-C (II) was collected with 5 mL of 0.9% NaCl–0.04 M EDTA, pH 7.0 (NaCl–EDTA) solution and centrifuged at 1600 rpm (IEC B-20 with IEC 870 rotor) for 5 min. This was repeated two more times. The cells were then suspended in NaCl–EDTA (total volume 3 mL), and H_2O (18 mL) was added in order to facilitate the destruction of erythrocytes and platelets. The cell suspension was cooled at 0 °C for 1 min, and then 3.6% NaCl (2 mL) was rapidly added. The cells were collected by centrifugation at 2000 rpm for 10 min. The cells were then washed with 10 mL of NaCl–EDTA three times.

Uptake of ara-C. The cells were incubated in RPMI-1640 with glutamine at 37 °C for 1 h to restore their high-energy metabolites. After the viable cells were counted by Trypan blue exclusion, the cells were incubated with [5-^{3}H]ara-C (5 μCi, Amersham) in the medium (total volume 10 mL, 0.1 mM of ara-C) at 37 °C for 60 min. Aliquots (0.2 mL) of the incubating mixture at the appropriate times were removed, and the radioactivity in the cells was measured as described previously.[33]

Deoxycytidine Kinase Activity. This was determined by a modification of the reported procedure.[34] Each mixture (pH 8.0) contained 0.5 mL of 48 mM Tris·HCl, 10–20 mM $MgCl_2$, 0.1 mM dithiothreitol, 5–10 mM ATP, 1 mM [5-^{3}H]deoxycytidine (4 μCi/μmol), 9 mM NaCl, 2.4 mM EDTA, and cell extract (0.1 and 0.2 mL) was incubated at 37 °C for 30 min. Aliquots (0.1 mL) at 1, 15, and 30 min were mixed first with 95% EtOH (0.1 mL) and then with H_2O (4.8 mL). The nucleotides were absorbed in DE82 (Cl^-) circles, and the radioactivity in the circles was counted.

Antitumor Activity in Vivo. DBA/2J mice in group of six (wt 20–30 g) were inoculated ip with 1×10^5 L1210/ara-C (I) or L1210/ara-C (II) lymphoid leukemia,[35] and a sonicated solution[6] of the conjugates or suspension of the reference prodrugs of Table III in 0.9% NaCl containing 0.5% Tween 80 was given ip as reported earlier.[6] Each drug was tested over a wide range of doses. The active dose ranges are those giving ILS values of ≥25%. Optimum doses are those producing the greatest increase in life span in the designated treatment schedule.

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Registry No. **1**, 69483-93-8; **2**, 115290-75-0; **3**, 115290-76-1; **4**, 30334-71-5; **5**, 6076-30-8; **6**, 40290-32-2; **7**, 7091-44-3; **8**, 58560-71-7; **9**, 5129-68-0; 1,2-isopropylidene-*sn*-glycerol, 22323-82-6;

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1,2-isopropylidene-3-*O*-benzyl-*sn*-glycerol, 16495-03-7; 3-*O*-benzyl-*sn*-glycerol, 56552-80-8; palmitoyl chloride, 112-67-4; 1,2-*O*-dipalmitoyl-3-*O*-benzyl-*sn*-glycerol, 30403-51-1; *rac*-3-*O*-benzylglycerol, 13071-59-5; 1,2-*O*-isopropylidene-3-*O*-palmitoyl-*sn*-glycerol, 57416-03-2; 3-*O*-palmitoyl-*sn*-glycerol, 5309-46-6; 1-*O*-trityl-3-*O*-palmitoyl-*sn*-glycerol, 30563-15-6; 1-*O*-trityl-2,3-*O*-dipalmitoyl-*sn*-glycerol, 30563-16-7; ara-CMP morpholidate, 69467-87-4.

Synthesis and Antiviral Evaluation of 6′-Substituted Aristeromycins: Potential Mechanism-Based Inhibitors of *S*-Adenosylhomocysteine Hydrolase[1,2]

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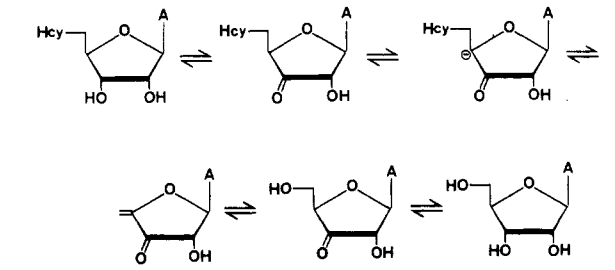
New carbocyclic adenosine analogues substituted at the 6′-position with fluorine, hydroxyl, methylene, or hydroxymethyl have been synthesized as potential mechanism-based inhibitors of *S*-adenosylhomocysteine (AdoHcy) hydrolase. The synthetic routes began with a functionalized (±)-azidocyclopentane **2**, which was elaborated to the adensoine analogue, or with functionalized cyclopentane epoxides **11**, **20**, and **27**, which were opened directly with adenine in the presence of base. The 6′α-fluoro (**24**), 6′β-fluoro (**10**), and 6′-methylene (**30**) carbocyclic adenosine analogues were potent inhibitors of AdoHcy hydrolase. None of the compounds displayed significant activity against herpes simplex virus type 1 or type 2, but several demonstrated potent inhibition of vaccinia virus replication.

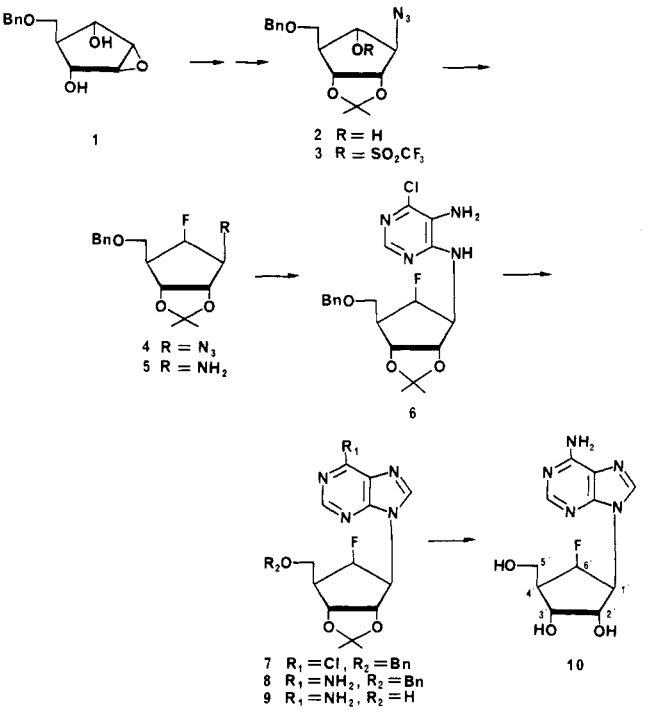
The antiviral activities of the carbocyclic adenosine analogues have been shown to be due, at least in part, to their inhibition of *S*-adenosylhomocysteine (AdoHcy) hydrolase.[6] For example, aristeromycin (the carbocyclic analogue of adenosine) shows activity against vaccinia virus[7] and inhibits AdoHcy hydrolase with a K_i of 5 nM.[8] Neplanocin A also inhibits vaccinia virus and has a K_i of 8 nM,[9] while the carbocyclic analogue of 3-deazaadenosine inhibits the growth of reo, measles, parainfluenza, vesicular stomatitis, herpes simplex-1, and vaccinia viruses[10,11] and displays a K_i value of 1 nM.[10] This relationship can be ratonalized by the fact that many viruses rely upon *S*-adenosylmethionine-dependent methylations to provide the methylated 5′-cap structure on their mRNAs. Since *S*-adenosylhomocysteine is a product and feedback inhibitor of these methylations, its accumulation via the inhibition of AdoHcy hydrolase will curtail viral mRNA capping. This, in turn, leads to inhibition of virus replication.[6b,c]

The mechanism by which AdoHcy hydrolase catalyzes the hydrolysis of AdoHcy to adenosine and homocysteine has been elegantly elucidated.[12–15] As shown in Scheme I, the first step involves oxidation of the 3′-hydroxyl group of AdoHcy by enzyme-bound NAD followed by β-elimi-

Scheme I



Scheme II



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