Nucleoside Conjugates. 14. Synthesis and Antitumor Activity of 1-β-D-Arabinofuranosylcytosine Conjugates of Ether Lipids with Improved Water Solubility[1]

Chung Il Hong,*,† Alexander Nechaev,† Alan J. Kirisits,† Rakesh Vig,† Sek-Wen Hui,‡ and Charles R. West†

Departments of Neurosurgery and Biophysics, Roswell Park Cancer Institute, Buffalo, New York 14263

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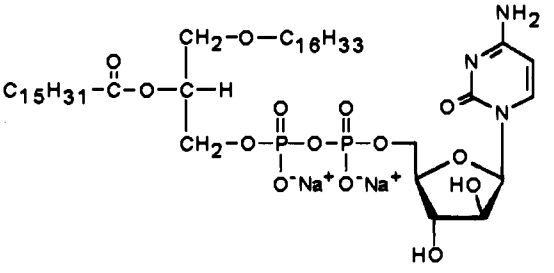
A series of *ara*-CDP-*rac*-1-*O*-alkyl-2-*O*-acylglycerols (**9a-f**), analogues of highly active *ara*-CDP-*rac*-1-*O*-hexadecyl-2-*O*-palmitoylglycerol (**1**) and Cytoros[2] (**2**), was prepared, and solubility, lipophilicity, and structure–activity relationships of these conjugates were investigated. Conjugates **9a-f** containing *sn*-1 alkyl ($<C_{16}$) and *sn*-2 fatty acyl ($<C_{14}$) and *sn*-1 alkyl ($<C_{14}$) and *sn*-2 fatty acyl ($<C_{16}$) substituents of the glycerol were water-soluble by shaking, while those with the *sn*-1 alkyl ($>C_{16}$) and the *sn*-2 fatty acyl ($>C_{16}$) such as conjugate **1** were sparingly soluble. Conjugates **9a-c,e** were almost completely solubilized in water by shaking. However, a large portion of conjugates **9d** and **9f** in water by shaking exist in micelles with mean diameters ranging 7.0–55.2 nm. The partition coefficients (1-octanol/PBS) of the water-soluble conjugates were about 9–18 times greater than that of *ara*-C. A single dose (300 mg/kg) of conjugates **9d** and **9f** produced a significant increase in life span (ILS 206 to >543%) with 17–67% long-term survivors (>45 days) in mice bearing ip-implanted L1210 lymphoid leukemia. These results were comparable to those of the previous conjugate **1** and Cytoros (**2**). In contrast, conjugates **9a-c,e** at single doses were less effective (ILS 69–178% with no long-term survivors). However, two (qd, 1, 7) or three (qd 1, 5, 9) divided doses of these conjugates were found to be as effective as a single dose of the previous conjugates. The three divided doses (150 mg/kg per day) of conjugates **9d**, **9e**, and **9f** produced a remarkable antitumor activity in L1210 leukemic mice (ILS >350% with >50% long-term survivors). Because of the convenient formulation and the significant antitumor activities, the water-soluble conjugates **9d**, **9e**, and **9f** warrant further investigation.

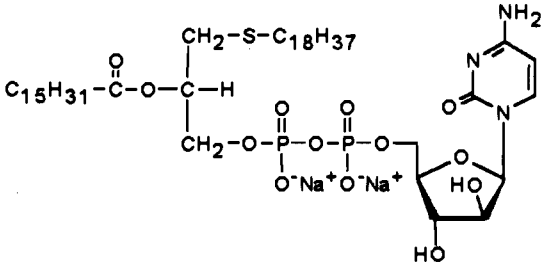
Micelle-forming 1-β-D-arabinofuranosylcytosine (*ara*-C)[2] conjugates of biologically active ether (1-*O*-alkyl) and thioether (1-*S*-alkyl) phospholipids have demonstrated a superior antitumor activity against both animal leukemia[3–7] and solid tumor models.[8–12] Among them, *ara*-CDP-*rac*-1-*O*-hexadecyl-2-*O*-palmitoylglycerol (1) and Cytoros (**2**) (Chart 1) are highly active.[5–15] However, water solubility of conjugates **1** and **2** was poor (0.13–2 mM) due to the lipophilic nature of the compounds. Previously, the water-soluble analogues of Cytoros (**2**), *ara*-C conjugates of thioether phospholipids, were synthesized by substituting the thioglycerol side chains with a variety of *sn*-1 alkyl ($<C_{18}$) and *sn*-2 fatty acyl ($<C_{14}$) substituents.[16] In contrast to Cytoros, the water-soluble conjugates were schedule-dependent for antitumor activity against L1210 lymphoid leukemia in mice.[16]

As an extension of our previous work, we have synthesized a series of water-soluble analogues of conjugate **1** with *sn*-1 alkyl (C_{12-16}) and *sn*-2 fatty acyl (C_{12-16}) substituents of the glycerol moiety. This paper describes the synthesis of these conjugates and their water solubility, lipophilicity, micelle formation, particle sizes in water solution, and antitumor activity against L1210 lymphoid leukemia in mice.

Chart 1. Chemical Structure of Conjugates 1 and 2



1



2 (Cytoros)

Chemistry

Scheme 1 describes the synthesis of the ether lipid intermediates and the conjugates. *rac*-1-*O*-Alkylglycerols (**3**) were prepared by alkylation of the alcohol function of 1,2-*O*-isopropylidene-*rac*-glycerol with alkyl

* To whom correspondence should be addressed.
† Department of Neurosurgery.
‡ Department of Biophysics.
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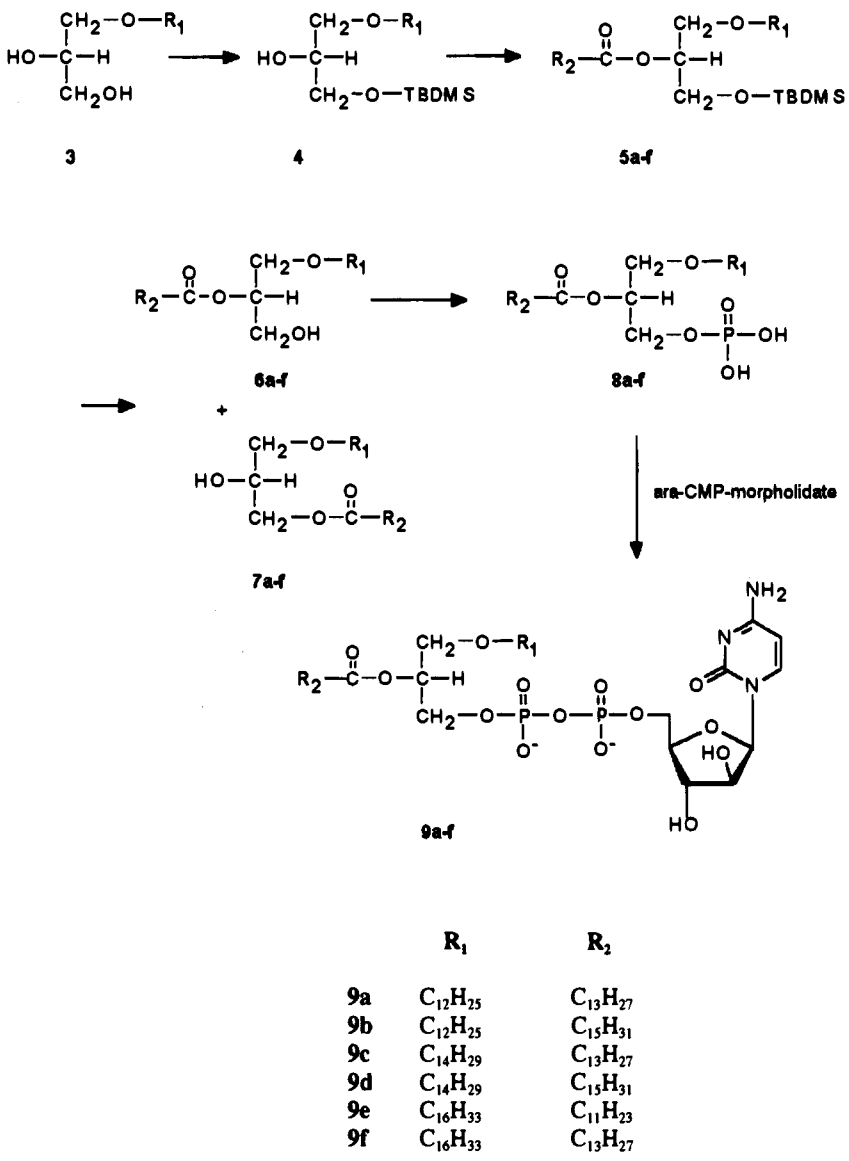
Scheme 1



	R_1	R_2
9a	$C_{12}H_{25}$	$C_{13}H_{27}$
9b	$C_{12}H_{25}$	$C_{15}H_{31}$
9c	$C_{14}H_{29}$	$C_{13}H_{27}$
9d	$C_{14}H_{29}$	$C_{15}H_{31}$
9e	$C_{16}H_{33}$	$C_{11}H_{23}$
9f	$C_{16}H_{33}$	$C_{13}H_{27}$

bromide and NaH.[17] The primary alcohol functions of the 1-*O*-alkylglycerols (**3**) were then protected with *tert*-butyldimethylsilyl chloride in the presence of imidazole and DMF. *rac*-1-*O*-Alkyl-3-*O*-(*tert*-butyldimethylsilyl)-glyerols (**4**) were then acylated with acyl chloride and pyridine, and the resulting compounds (**5a-f**) were purified by crystallization from a large amount of boiling 95% EtOH. The TBDMS group was removed by treatment of **5a-f** in HOAc with tetrabutylammonium fluoride in THF at 5–10 °C first and then at room temperature. *rac*-1-*O*-Alkyl-2-*O*-acylglcerols (**6a-f**) were obtained in 58–79% yield. Acyl migration occurred even during the crystallization in 95% EtOH. The thermodynamically more stable isomers, *rac*-1-*O*-alkyl-3-*O*-acylglycerols (**7a-f**), were obtained also in 30% yield. *rac*-1-*O*-Alkyl-2-*O*-acylglycerols (**6a-f**) were phosphorylated with $POCl_3$ and Et_3N at 0–5 °C as outlined previously,[7] and the resulting phosphates (**8a-f**) were purified by successive crystallizations with hexanes and Et_2O. The crude phosphates were then condensed with *ara*-CMP morpholidate[18] in pyridine, and the conjugates (**9a-f**) were obtained in 24–49% yield (Table 1). Structures were verified by elemental analysis and ^1H NMR and UV spectrometry (Tables 1 and 2).

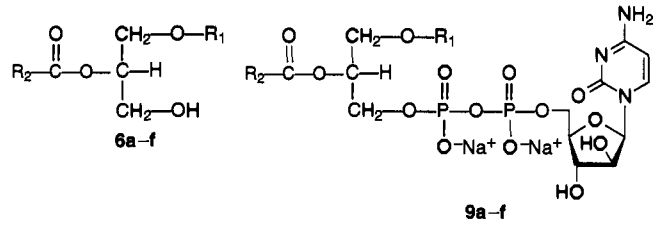
Water Solubility

The water solubilities of the conjugates in sterile water for injection, USP at room temperature, are listed in Table 2. Conjugates **9a-f** with *sn*-1 alkyl ($<C_{16}$) and *sn*-2 fatty acyl ($<C_{14}$) or with *sn*-1 alkyl ($<C_{14}$) and *sn*-2 fatty acyl ($<C_{16}$) substituents of the glycerol moiety were soluble in water (concentration >43 mM), while **1** with the *sn*-1 alkyl (C_{16}) and the *sn*-2 fatty acyl (C_{16}) was sparingly soluble in water (concentration 0.13 mM). In other words, conjugates with a total of less than 30 carbons on *sn*-1 and *sn*-2 of the glycerol were soluble in water.

Lipophilicity

Partition coefficients (*P*) were determined for all conjugates using a mixture of 1-octanol and phosphate-buffered saline solution (PBS), pH 7.4, at room temperature. The results are shown in Table 2. *P* values for the water-soluble conjugates **9a-f** ranged from 0.114 to 0.233, while that of the water-insoluble **1** was 0.595. Thus, the increase in lipophilicity of the water-soluble conjugates was about 9–18-fold as compared to that of *ara*-C ($p = 0.013$), while that of the water-insoluble was 46-fold.

Table 1. Structures and Physical Properties of Ether Lipids and *ara*-C Conjugates



compd	R_1	R_2	yield, %	mp, °C	formula	analysis
6a	$C_{12}H_{25}$	$C_{13}H_{27}$	71	54–54.5	$C_{29}H_{58}O_4$	C, H
6b	$C_{12}H_{25}$	$C_{15}H_{31}$	58	54–55	$C_{31}H_{62}O_4$	C, H
6c	$C_{14}H_{29}$	$C_{13}H_{27}$	61	60–61	$C_{31}H_{62}O_4$	C, H
6d	$C_{14}H_{29}$	$C_{15}H_{31}$	67	63–64	$C_{33}H_{66}O_4$	C, H
6e	$C_{16}H_{33}$	$C_{11}H_{23}$	79	56–57	$C_{31}H_{62}O_4$	C, H
6f	$C_{16}H_{33}$	$C_{13}H_{27}$	71	55–56	$C_{33}H_{66}O_4$	C, H
9a	$C_{12}H_{25}$	$C_{13}H_{27}$	39	186–187	$C_{38}H_{69}N_3O_{14}P_2 \cdot 2Na \cdot 2H_2O$	C, H, N
9b	$C_{12}H_{25}$	$C_{15}H_{31}$	49	182–183	$C_{40}H_{73}N_3O_{14}P_2 \cdot 2Na \cdot H_2O$	C, H, N
9c	$C_{14}H_{29}$	$C_{13}H_{27}$	35	188–189	$C_{40}H_{73}N_3O_{14}P_2 \cdot 2Na \cdot H_2O$	C, H, N
9d	$C_{14}H_{29}$	$C_{15}H_{31}$	44	190–191	$C_{42}H_{77}N_3O_{14}P_2 \cdot 2Na \cdot 2.5H_2O$	C, H, N[a]
9e	$C_{16}H_{33}$	$C_{11}H_{23}$	32	191–192	$C_{40}H_{73}N_3O_{14}P_2 \cdot 2Na \cdot H_2O$	C, H, N
9f	$C_{16}H_{33}$	$C_{13}H_{27}$	24	180–181	$C_{42}H_{77}N_3O_{14}P_2 \cdot 2Na \cdot H_2O$	C, H, N

[a] N: calcd, 4.20; found, 3.68.

Table 2. Physical Data of the Conjugates

compd	no. of carbons $R_1 + R_2CO$	water solubility (mM)[a]	partition coefficients (P)[b]	UV_{max}, nm ($\epsilon \times 10^{-3}$) neutral	acid	base
1	32	0.13	0.595	271 (5.96)	282 (7.95)	270 (5.52)[c]
9a	26	>146	0.114	272 (8.43)	282 (14.73)	272 (6.40)
9b	28	>131	0.233	272 (8.84)	282 (9.91)	272 (8.57)
9c	28	>121	0.202	272 (10.65)	282 (14.96)	272 (9.68)
9d	30	>43	0.170	272 (8.55)	282 (10.22)	272 (9.18)
9e	28	>132	0.194	272 (8.91)	282 (12.26)	272 (9.17)
9f	30	>107	0.224	272 (8.72)	282 (9.69)	272 (8.51)

[a] Determined by UV absorption at 272 nm. [b] Partition coefficients (P) in 1-octanol/PBS (pH 7.4) at 25 °C. P value for *ara*-C = 0.013. [c] UV_{max} data from ref 3.

Table 3. Mean Particle Sizes of Water-Soluble *ara*-C Conjugates of Ether Phospholipids

compd[b]	method of formulation	volume-weighed nonlinear multimodal analysis[a] mean diameter, nm (vol %) peak no. 1	peak no. 2
1	sonication	5.9 (88)	41.5 (12)
9a	shaking	2.3 (96)	7.8 (4)
9b	shaking	2.2 (100)	
9c	shaking	2.2 (98)	8.6 (2)
9d	shaking	2.2 (73)	7.0 (26)
	sonication	2.2 (98)	11.9 (2)
9e	shaking	2.2 (100)	
9f	shaking	10.2 (77)	55.2 (22)
	sonication	2.2 (98)	37.8 (2)

[a] Analyzed using photon correlation spectroscopy on NICOMP 370 Submicron Particle Sizer with ZERO-OFF and peak at around 2 nm is an artifact.[19] Peaks with <2% were deleted. [b] Average particle sizes of Cytoros (**2**) in sonicated solution were 7.4 (98%) and 37.8 nm (2%).

Particle Size

Mean diameter of particles of conjugates **1**, **2**, and **9a-f** in water solution by either sonication or shaking were determined by volume-weighed nonlinear multimodal analysis using photon correlation spectroscopy[19] on a NICOMP 370 Submicron Particle Sizer[20] (NICOMP Particle Sizing Systems, Santa Barbara, CA) (Table 3). Mean particle sizes of micelle-forming conjugate **1** and Cytoros (**2**) in the sonicated solution were mainly 5.9 and 7.4 nm, respectively. Mean particle sizes of water-soluble conjugates **9a-c,e** in the shaken solution were mainly (>96%) 2.2–2.3 nm. Since the peak around 2 nm recorded by the instrument is an artifact,[20] most of them are solubilized in water. Conjugates **9a** and **9c** in the shaken solution also contained particles of 7.8 (4%) and 8.6 nm (2%), respectively, which represented micelles. Conjugates **9d** and **9f** were almost completely solubilized in the sonicated solution (particle size 2 nm, 98%) and a small portion of them existed in micelles, which was confirmed from the negative-staining electron micrograph.[21] Particle sizes of conjugate **9d** in the shaken solution were 2.2 (73%) and 7.0 nm (26%), indicating that more than 26% of **9d** existed in micelles. Conjugate **9f** in the shaken solution also existed mainly (99%) in micelles with mean particle sizes ranging from 10.2 to 55.2 nm.

Antitumor Activity

In Table 4, conjugates 1 and **9a-f** were compared for in vivo antitumor activity against ip implanted L1210 lymphoid leukemia in DBA/2Ros mice according to the procedures outlined in the NCI protocols[22] with some modifications including inoculation of 1×10^6 cells as opposed to 1×10^5 cells and a 45-day observation period. Optimum single doses (ip) of conjugates **9a-c,e** produced marginal activities with 69–128% ILS values and no long-term survivors (>45 days). However, two divided doses of **9b** and **9e** (200 mg/kg per day) given on days 1 and 7 improved their activities with the respective ILS values of 286% and >350%. Three divided doses of **9a**, **9b**, and **9e** (150 mg/kg per day) given on days 1, 5, and

Table 4. Antitumor Activity against Ip-Implanted L1210 Lymphoid Leukemia in Mice[a]

compd	treatment schedule, qd	optimal dose, mg (μmol)/kg per day	formulation[b]	survival days: range	survival days: median T/C[c]	% ILS[d]	45-day survivors
1	1	200 (203)	sonication	8 to >45	>28.0/8.0	>250	3/6
	1, 5, 9	150 (152)	sonication	21 to >45	32.5/8.0	306	3/6
9a	1	200 (222)	shaking	13–14	13.5/8.0	69	0/6
	1, 5, 9	150 (167)	shaking	25 to >45	>39.5/8.0	>394	3/6
9b	1	200 (216)	shaking	15–21	16.5/7.0	128	0/6
	1, 7	200 (216)	shaking	22–34	27.0/7.0	286	0/6
	1, 5, 9	150 (162)	shaking	5 to >45	>33.0/8.0	>313	3/6
9c	1	300 (437)	shaking	16–20	19.5/7.0	178	0/6
	1, 5, 9	150 (162)	shaking	5 to >45	18.0/8.0	125	1/6
9d	1	200 (209)	shaking	14 to >45	30.0/8.0	275	2/6
			sonication	15 to >45	24.5/8/0	206	1/6
	1	300 (314)	shaking	34 to >45	>45.0/8.0	>543	4/6
			sonication	18 to >45	24.5/8.0	206	2/6
	1, 7	300 (314)	shaking	13 to >45	14.5/7.0	107	1/6
	1, 5, 9	150 (157)	shaking	5 to >45	>36.0/8.0	>350	3/6
			sonication	22 to >45	>45.0/8.0	>463	5/6
9e	1	300 (323)	shaking	17–21	17.5/8.0	119	0/6
	1, 7	200 (215)	shaking	23 to >45	>36.0/8.0	>350	3/6
	1, 5, 9	150 (162)	shaking	25 to >45	>45.0/8.0	>463	5/6
9f	1	300 (314)	shaking	20 to >45	25.0/7.0	258	1/6
			sonication	24 to >45	>39.5/7.0	>464	3/6
	1, 5, 9	150 (157)	shaking	26 to >45	>45.0/8.0	>463	5/6
			sonication	12 to >45	>45.0/8.0	>463	4/6

[a] Each group of 6 DBA/2Ros mice (male, 20–29 g) received ip inoculation of 1×10^6 cells on day 0. Treatments (ip) were initiated on day 1. [b] Sonication: micelles by sonication; shaking: solution or micelles by shaking. (See text for detail). [c] Calculated based on survivors according to the NCI protocols.[22] [d] Increase in life span: (T/C − 1) × 100.

9 further improved their activities (ILS >313% and >50% long-term survivors). Particularly, **9e** in both the two and the three divided doses was highly active and resulted in 50% and 83% long-term survivors, respectively. Unlike conjugates **9a-c,e**, an optimum single dose (300 mg/kg) of **9d** and **9f** in both the shaken and the sonicated solutions exhibited a significant antitumor activity. Conjugate **9d** gave ILS values of 206 to >543% and 33–67% long-term survivors. Conjugate **9f** also produced ILS values of 258 to >543% and 17–67% long-term survivors. These results are comparable to that of the previous micelle-forming conjugate **1**. Conjugates **9d** and **9f** in multiple doses (qd 1, 5, 9, 150 mg/kg per day) were also highly active (ILS 350 to >463% and 50–83% long-term survivors).

Discussion

The technique of dynamic light scattering (DLS) or photon correlation spectroscopy has developed into a powerful and versatile tool for estimating the particle size distribution of fine-particle materials effective from a few nanometers (nm) to several micrometers (μm).[19] Ultrafine particles having mean diameters below 10–15 nm contain a rich assortment of systems of broad interest including surfactant micelles, reverse micelles (water-in-oil microemulsions), ultrafine colloidal silicas, proteins, peptides, and other macromolecules of biological significance. The previous conjugates **1** and **2** (Cytoros) form micelles when the water suspensions are sonicated.[3,10] In Table 3, mean particle sizes of these micelles analyzed using photon correlation spectroscopy[19] are 5.9–41.5 nm, which are comparable to those measured from the freeze-fracture electron micrograph of Cytoros (**2**).[10]

Conjugates **9a-f** with *sn*-1 alkyl (<C_{16}) and *sn*-2 fatty acyl (<C_{16}) substituents of the glycerol are water-soluble (Table 2). Analysis of mean particle sizes of conjugates **9a-c,e** in water solution indicate that they are completely solubilized in water (Table 3). Conjugates **9d** and **9f** in the sonicated solution also exist mainly in solution. However, a large portion of these conjugates in the shaken solution exists in micelles with mean diameters ranging from 7.0 to 55.2 nm.

Conjugates **9d** and **9f** exhibited a significant antitumor activity with a single dose treatment of ip-inoculated L1210 leukemic mice, while conjugates **9a-c,e** with a single dose produced somewhat marginal activity (Table 4). However, the latter conjugates with the three divided doses gave a comparable activity to the former with a single dose. Particularly, conjugate **9e** with both the two and the three divided doses was highly active. Conjugates **9d** and **9f** in both the shaken and the sonicated solutions given in single or divided doses were found to be highly active.

Conjugates **9d** and **9f**, which are soluble in water and form micelles by shaking, have the pharmacologically favorable properties as demonstrated previously with the *ara*-C conjugates of thioether phospholipids.[16] In fact, administration of micellar solution of Cytoros (**2**) into L1210 leukemic mice gave a greater intracellular retention of *ara*-CTP than that resulting from *ara*-C.[10,14] Besides increased *ara*-CTP retention, other possible favorable properties of micellar solution of the conjugates are rapid interaction with serum lipoproteins,[23] the release of more drug, the same amount of drug over a longer interval, and release of drug at a more constant rate than if micelles are absent.[24]

The increased solubility in a micellar solution of a water-insoluble or sparingly soluble organic substance is a property which has been applied to drug formulation. It has been demonstrated previously that combination chemotherapy with nitrosoureas or etoposide solubilized in micelles of Cytoros (**2**) and its derivatives produce a synergy.[11,15,25] Therefore, in addition to the use of the water-soluble and micelle-forming conjugates as an effective anticancer drug, they can be utilized as

both the drug and solubilizer in combination chemotherapy with other lipophilic anticancer agents.

In summary, conjugates **9d**, **9e**, and **9f** warrant further investigation because of their convenient formulation, significant antitumor activity, and potential utilization of combination chemotherapy.

Experimental Section

Synthesis. Melting points were taken on Mel-Temp capillary melting point apparatus and are uncorrected. ^{1}H NMR spectra were recorded on a Varian Associates 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. Average particle sizes of the conjugates in water solution by either sonication or shaking were determined by dynamic light scattering technique using a NICOMP 370 Submicron Particle Sizer (NICOMP Particle Sizing Systems). AG1-X8 (Bio-Rad) and [(diethylamino)ethyl]-cellulose (DE-52, Whatman) were used for column chromatography. Evaporations were carried out on a rotary evaporator under reduced pressure applied by an Aspirator A-3S (Wheaton) or a vacuum pump with a bath temperature of under 30 °C. TLC was performed on a glass plates coated a 0.25-mm layer of silica gel PF-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 *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 detected with a modified Dittmer-Lester spray.[26] Elemental analyses were performed by Galbraith Laboratories, Knoxville, TN, and Robertson Laboratory, Madison, NJ. When analyses are reported only by the elemental symbols, results are within ±0.4% of the theoretical values including given numbers of H_2O of hydrations unless noted otherwise. The presence of H_2O as indicated by elemental analysis was verified by ^{1}H NMR.

Ara-CMP,[27] *ara*-CMP morpholidate,[18] and *rac*-1-*O*-hexadecylglycerol (**3**, $R_1 = C_{14}H_{29}$)[3] were prepared by a literature procedure.

***rac*-1-*O*-Tetradecylglycerol (3, $R_1 = C_{14}H_{29}$).** To a mixture of Solketal (2,2-dimethyl-1,3-dioxolane-4-methanol) (66.1 g, 0.50 mol) and 1-bromotetradecane (160.8 g, 0.58 mol) in 800 mL of DMF was added 60% NaH in oil dispersion (26 g, 0.65 mol) in three portions at room temperature for a period of 1 h. The mixture was stirred at room temperature for 1 day, and then MeOH (20 mL) was added to the reaction mixture in order to destroy the excess NaH. This was then poured into ice water (1.5 L), and the mixture was extracted with hexanes (700 mL × 3). The organic extracts were pooled and evaporated to dryness, and the residue was refluxed in 10% HCl (1 L) for 30 min. After standing at room temperature overnight, the resulting white flaky crystals were filtered and washed with MeOH: yield 122 g. The additional product (5.7 g) was obtained by cooling the filtrate after extracting with hexanes (300 mL). The products were recrystallized from diethyl ether: total yield 99.7 g (69%); mp 57–58 °C; ^{1}H NMR ($CDCl_3$) δ 0.85 (3, t, J = 6 Hz, CH_3), 1.27 (22, s, $(CH_2)_{11}$), 1.53 (2, m, OCH_2CH_2), 3.37–3.67 (6, m, CH_2OCH_2, 3-CH_2), 3.80 (1, m, 2-CH). Anal. ($C_{17}H_{36}O_3$) C, H.

rac-1-*O*-Dodecylglycerol (**3**, $R = C_{12}H_{25}$) was prepared in an analogous manner: yield 60.4%; mp 49–50 °C; ^{1}H NMR ($CDCl_3$) δ 0.87 (3, t, J = 6 Hz, CH_3), 1.27 (18, s, $(CH_2)_9$), 1.53 (2, m, OCH_2CH_2), 3.33–3.63 (6, m, CH_2OCH_2, 3-CH_2), 3.80 (1, m, 2-CH). Anal. ($C_{15}H_{32}O_3$) C, H.

***rac*-1-*O*-Tetradecyl-3-*O*-(*tert*-butyldimethylsilyl)glycerol (4, $R_1 = C_{14}H_{29}$).** A mixture of **3** ($R_1 = C_{14}H_{29}$) (57.7 g, 0.20 mol), *tert*-butyldimethylsilyl chloride (33.2 g, 0.22 mol), imidazole (30.0 g, 0.44 mol), and DMF (300 mL) was stirred at room temperature for 1 day. The solvent was evaporated to dryness in vacuo at 70 °C, and the residue was partitioned between H_2O and Et_2O (500 mL each). The organic layer was dried over Na_2SO_4 and then evaporated to dryness. The oily residue was further evaporated by using a high vacuum at 70 °C. The crude product, essentially homogeneous by TLC, weighed 80 g (100%) and was used for the next step without further purification.

Other *rac*-1-*O*-alkyl-3-*O*-(*tert*-butyldimethylsilyl)glycerols (**4**) were prepared by using an analogous procedure: yield >83%.

***rac*-1-*O*-Tetradecyl-2-*O*-palmitoyl-3-*O*-(*tert*-butyldimethylsilyl)glycerol (5d).** To a mixture of the above product (40.3 g, 0.10 mol), anhydrous pyridine (10 mL), and toluene (300 mL) was added dropwise palmitoyl chloride (30.2 g, 0.11 mol) at room temperature, and the mixture was stirred at room temperature for 2 days. The mixture was then partitioned between Et_2O and H_2O (200 mL each). The organic layer was washed with 0.5 N H_2SO_4, saturated $NaHCO_3$, and H_2O (100 mL each) and then evaporated to dryness. The residue was dissolved in boiling 95% EtOH, and the solution was cooled at room temperature. The oily product was separated by decantation and washed with 95% EtOH. The product was dried in vacuo (60.3 g, 94%) and was essentially homogeneous by TLC and used for the next step without further purification.

Other *rac*-1-*O*-alkyl-2-*O*-acyl-3-*O*-(*tert*-butyldimethylsilyl)-glycerols (**5a-c,e,f**) were prepared in an analogous manner: yield >95%.

***rac*-1-*O*-Tetradecyl-2-*O*-palmitoylglycerol (6d).** To a mixture of above product (60.3 g, 94 mmol) in HOAc (13.2 mL) was added dropwise 1 M tetrabutylammonium fluoride in THF (132 mL) for a period of 1 h at 5–10 °C, and the mixture was stirred at room temperature for 2 days. After cooling at 0–5 °C overnight, the solid was filtered and washed with ice-cold 95% EtOH: yield 33 g (67%). The analytical sample was crystallized from 95% EtOH: mp 63–64 °C; ^{1}H NMR δ 0.90 (6, t, J = 6 Hz, 2 CH_3), 1.30 (48, s, $(CH_2)_{11}$, $(CH_2)_{13}$), 1.52 (2, m, OCH_2CH_2), 2.32 (2, t, J = 7.5 Hz, CH_2CH_2CO), 3.40 (2, t, J = 6 Hz, CH_2OCH_2), 3.57 (2, d, J = 5 Hz, 1-CH_2), 3.77 (2, d, J = 5 Hz, 3-CH_2), 4.97 (1, quintet, J = 5 Hz, 2-CH).

Other *rac*-1-*O*-alkyl-2-*O*-acylglycerols (**6a-c,e,f**) were prepared in analogous manner: yield 58–79%.

***rac*-1-*O*-Tetradecyl-2-*O*-palmitoylglycerol 3-Phosphate (8d).** To a ice-cold mixture of $POCl_3$ (9.2 g, 60 mmol) and hexanes (25 mL) was added dropwise triethylamine (6.17 g, 60 mmol) in hexanes (25 mL). To this mixture was added dropwise a solution of the above product (21.1 g, 40 mmol) in toluene (500 mL) at 0–5 °C over a period of 1 h, and the mixture was stirred at room temperature overnight. Water (100 mL) was added to the mixture followed by stirring at room temperature for 1 h. The mixture was partitioned between Et_2O (500 mL) and H_2O (200 mL). The organic layer was dried over Na_2SO_4 and evaporated to dryness. The residue was crystallized from Et_2O at 0–3 °C and then recrystallized repeatedly from Et_2O at 10 °C: yield 15.2 g (63%); mp 56–58 °C wet 60–80 °C slowly melt. The product was essentially homogeneous by TLC and used for the condensation without further purification and elemental analysis.

Other *rac*-1-*O*-alkyl-2-*O*-acylglycerol 3-phosphates (**8a-c,e,f**) were prepared in an analogous manner, and the crude phosphates were used for the condensation.

***ara*-CDP-*rac*-1-*O*-tetradecyl-2-*O*-palmitoylglycerol (9d).** The above phosphate **8d** (11.0 g, 22 mmol) was dried azeotropically with pyridine twice and mixed with *ara*-CMP morpholidate[18] (7.5 g, 11 mmol) followed by coevaporation with pyridine three times. The dried mixture was then mixed with anhydrous pyridine (500 mL) and stirred at room temperature for 7 days. After removal of the solvent in vacuo, the residue was coevaporated with toluene to remove the residual pyridine. The residue was dissolved in 1000 mL of $CHCl_3$–95% EtOH–H_2O (2:4:1) and then was shaken with 0.5 N HCl (100 mL). The aqueous layer was extracted with $CHCl_3$ (100 mL). The combined organic layers were evaporated to dryness, and the residue was dissolved in 500 mL of $CHCl_3$–95% EtOH–H_2O (2:4:1). The solution was applied to a DE-52 (AcO^-) column (4 × 35 cm) equilibrated with the same solvent. The column was eluted with $CHCl_3$–95% EtOH–H_2O (2:4:1) (1.5 L) and then with 0.02 M NaOAc in the same solvent. The 0.02 M NaOAc fractions between 1750 and 3250 mL were evaporated to a small volume (20 mL) and mixed with acetone (200 mL). The mixture was cooled at 0–5 °C overnight, and the product (Na salt) was filtered, washed with acetone, and dried in

vacuo: yield 4.87 g (44%); mp 190–192°C; ^{1}H NMR ($CDCl_3$–CD_3OD–D_2O, 2:3:1) δ 0.87 (6, n, 2 CH_3), 1.30–1.70 (50, m, $(CH_2)_{12}$, $(CH_2)_{13}$), 2.27 (2, t, J = 7.5 Hz, CH_2CH_2CO), 3.40 (2, m, CH_2OCH_2), 3.57 (2, t, J = 5 Hz, 1-CH_2), 3.63 (2, t, J = 5 Hz, 3-CH_2), 4.12–4.73 (5, m, H-2′, H-3′, H-4′, H-5′), 5.03 (1, m, 2-CH), 5.95 (1, d, J = 7.5 Hz, cytosine H-5), 6.17 (1, d, J = 5 Hz, H-1′), 7.83 (1, d, J = 7.5 Hz, cytosine H-6).

The conjugates **9a-c,e,f** in Table 1 were prepared in an analogous manner.

Water Solubility. The conjugate (160 mg) in 1 or 5 mL of sterile water for injection, USP, was shaken at room temperature using a Gyrotary Water Bath Shaker model G76 (New Brunswick Scientific) with a speed setting at 6 for 2 h, and the solution or suspension was filtered through a membrane filter (0.22 μm). The concentration of the free conjugate in the filtrate was checked by quantitative UV at 273 nm.

The conjugate in water suspension was sonicated using Branson Sonifer Cell Disrupter 200 (Branson Power Co.) at output control at 5 for 2 min (temperature 20–60 °C), and the clear solution was filtered through a membrane filter (0.22 μm).

Partition Coefficient Measurements. 1-Octanol/aqueous phase partition coefficients were determined at room temperature using the shake-flask procedure described previously.[28] The UV absorbance of both phases were measured at 273 nm. The partition coefficients were calculated from the ratio of the absorbance between the 1-octanol and aqueous phases.

Determination of Particle Sizes. Mean particle sizes of the conjugates in water solution by shaking or sonication were determined using photon correlation spectroscopy[19] on a NICOMP 370 Submicron Particle Sizer (NICOMP Particle Sizing Systems, Santa Barbara, CA) with very-high-power argon laser (500 nm, Coherent Inova 70).[20]

Biological Studies. Antitumor Activity in Vivo. DBA/2Ros male mice in groups of 6 (wt 20–29 g) were inoculated ip with 1×10^6 (or ic with 1×10^5) L1210 lymphoid leukemia cells,[20] and a shaken or a sonicated solution of the conjugates was given ip as reported earlier.[10] Each drug was tested over a wide range of doses. The results from the optimal dose levels are shown in Table 4.

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(2) The abbreviations used are as follows: *ara*-C, 1-β-D-arabinofuranosylcytosine; *ara*-CMP, *ara*-C 5′-monophosphate; *ara*-CDP, *ara*-C 5′-diphosphate; *ara*-CTP, *ara*-C 5′-triphosphate; Cytoros, *ara*-CDP-*rac*-1-*S*-octadecyl-2-*O*-palmitoyl-1-thioglycerol (*ara*-CDP-β-palmitoyl-DL-thiobatyl alcohol); PBS, phosphate-buffered saline (pH 7.4); TBDMS, *tert*-butyldimethylsilyl; ILS, increase in life span; ip, intraperitoneally.

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