# Nucleoside Conjugates. 13. Synthesis and Antitumor Activity of 1-β-D-Arabinofuranosylcytosine Conjugates of Thioether Lipids with Improved Water Solubility[1]

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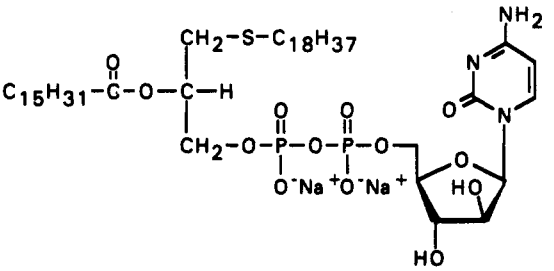
A series of *ara*-CDP-*rac*-1-*S*-alkyl-2-*O*-acyl-1-thioglycerols (3–12), analogues of highly active Cytoros[2] (1), was prepared, and solubility, lipophilicity, and structure–activity relationships of these conjugates were investigated. The conjugates with *sn*-1 alkyl ($<C_{18}$) and *sn*-2 fatty acyl ($<C_{14}$) substituents of the thioglycerol were water-soluble, while those with the *sn*-1 alkyl ($>C_{14}$) and the *sn*-2 fatty acyl ($>C_{16}$) were sparingly soluble. The latter formed micelles upon sonication. Conjugate 7 containing the *sn*-1 tetradecyl and the *sn*-2 palmitoyl ($C_{16}$) groups formed micelles by both sonication and shaking. The partition coefficients (1-octanol/PBS) of the water-soluble conjugates were about 20 times greater than that of *ara*-C. The water-insoluble showed a more than 40 times increase. A single dose of the micelle-forming conjugates 7 and 10 produced a significant increase in life span (ILS >421%) with 50% long-term survivors (>45 days) in mice bearing ip-implanted L1210 lymphoid leukemia. These results were comparable to those of previous micelle-forming conjugate 1 (Cytoros). In contrast, the water-soluble conjugates at single doses were less effective (ILS 81–386% with 0–33% long-term survivors). However, three divided doses of the water-soluble conjugates were found to be as effective as a single dose of micellar solution of the water-insoluble. The results indicate that conjugate 7 and most of the water-soluble derivatives warrant further investigation.

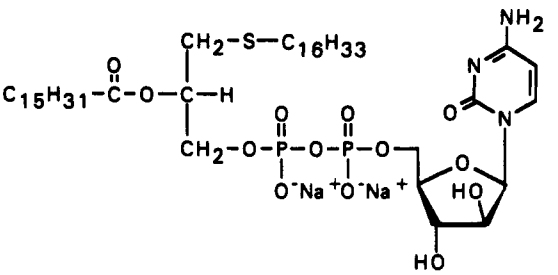
1-β-D-Arabinofuranosylcytosine (*ara*-C)[2] conjugates of biologically active thioether (1-*S*-alkyl) phospholipids have demonstrated a superior antitumor activity against both animal leukemia[3–6] and solid tumor models.[7–11] Among them, conjugates 1 (Cytoros) and 2 (Chart I) are highly active,[9–11] and particularly, Cytoros (1) has shown significant therapeutic effects on human colorectal[12] and PSN-1 pancreatic cancer xenografts in nude mice.[13] Due to the lipophilic thioglycerol moiety with *sn*-1 octadecyl and *sn*-2 palmitoyl groups, water-solubility of Cytoros (1) is very poor (0.2 mM). Thus, Cytoros has been formulated in micellar solution by sonication, and the micellar formulation is very stable at 0–3 °C.[9] In an attempt to improve water solubility of Cytoros and its analogues, a series of *ara*-C conjugates of thioether phospholipids with a variety of *sn*-1 alkyl ($C_{10-18}$) and *sn*-2 fatty acyl ($C_{12-18}$) substituents of the thioglycerol moiety has been synthesized.

This paper describes the synthesis of these conjugates and their water solubility, lipophilicity, micelle formation, and antitumor activity against L1210 lymphoid leukemia in mice.

**Chart I.** Chemical Structure of Conjugates 1 and 2




1 (Cytoros)

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## Chemistry

Conjugates 3–12 were prepared by condensation of *ara*-CMP morpholidate (19)[14] and the appropriate 1-*S*-alkylphosphatidic acids (18) in a manner reported previously (Scheme I).[6] A major advantage of the synthetic route was the synthesis of pure 1-*S*-alkylphosphatidic acids (18) without using column chromatography. *rac*-1-*S*-Alkyl-1-thioglycerols (13) were prepared by alkylation of the mercaptan of DL-1-thioglycerol with alkyl bromide and alcoholic potassium hydroxide.[15] The primary alcohol functions of the 1-*S*-alkyl-1-thioglycerols (13) were then protected with *tert*-butyldimethylsilyl chloride in the presence of imidazole and DMF. *rac*-1-*S*-Alkyl-3-*O*-(*tert*-butyldimethylsilyl)-1-thioglycerols (14) were then acylated with acyl chloride and pyridine, and the resulting compounds (15) were purified by crystallization from a large amount of boiling 95% EtOH. The TBDMS group was removed by treatment of *rac*-1-*S*-alkyl-2-*O*-acyl-3-*O*-(*tert*-butyldimethylsily)-1-thioglycerols (15) in HOAc with tetrabutylammonium fluoride in THF at 5–10 °C first and then at room temperature. *rac*-1-*S*-Alkyl-2-*O*-acyl-1-thioglycerols (16) were obtained in 52–70% yield. Acyl

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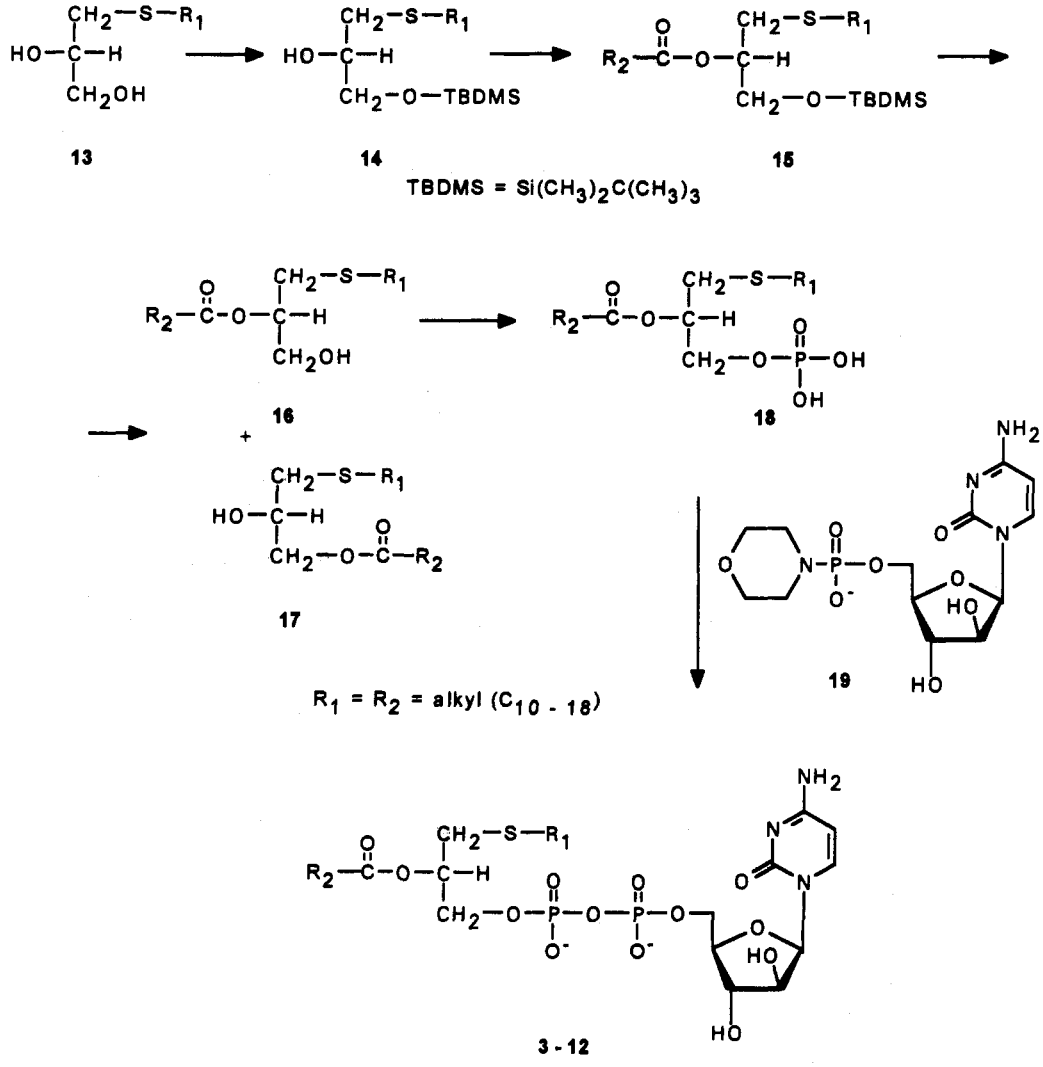
**Scheme I**




migration occurred even during the crystallization in 95% EtOH. The thermodynamically more stable isomers, *rac*-1-*S*-alkyl-3-*O*-acyl-1-thioglycerols (**17**), were obtained also in 30% yield. *rac*-1-*S*-Alkyl-2-*O*-acyl-1-thioglycerols (**16**) were phosphorylated with $POCl_3$ and $Et_3N$ at 0–5 °C as outlined previously,[6] and the resulting phosphates (**18**) were purified by successive crystallizations with hexanes and $Et_2O$. The crude phosphates were then condensed with *ara*-CMP morpholidate (**19**) in pyridine, and the conjugates were obtained in 14–42% yield (Table I). Structures were verified by elemental analyses and $^1H$ NMR and UV spectrometry (Tables I and II).

## Water Solubility

The water solubilities of the conjugates in sterile water for injection, USP at room temperature, are listed in Table II. Conjugates **3–6, 8, 9, 11,** and **12** with *sn*-1 alkyl ($<C_{18}$) and *sn*-2 fatty acyl ($<C_{14}$) substituents of the thioglycerol moiety were soluble in water (concentration >50 mM), while **1, 2, 7,** and **10** with the *sn*-1 alkyl ($>C_{14}$) and the *sn*-2 fatty acyl ($>C_{16}$) were sparingly soluble in water (concentration 0.2–1.5 mM). Among conjugates **7, 9,** and **11** with a total of 30 carbons on *sn*-1 and *sn*-2 of the thioglycerol, conjugate **7** containing a *sn*-2 palmitoyl ($C_{16}$) group was slightly soluble in water. The same trend was observed in conjugates **2** and **12** (total carbon 34), and conjugate **2** with the *sn*-2 palmitoyl substituent was sparingly soluble in water. In other words, the conjugates with more than total 30 carbons in the *sn*-1 and the *sn*-2 substituents and with a *sn*-2 fatty acyl ($>C_{16}$) group are sparingly soluble in water. These results indicate that the *sn*-2 fatty acyl substituent may affect solubility more than the *sn*-1 alkyl. Conjugates **1, 2, 7,** and **10** were formulated in micellar solution by sonicating the water suspension.[9] Conjugate **7** formed a micellar solution by both sonication and shaking.

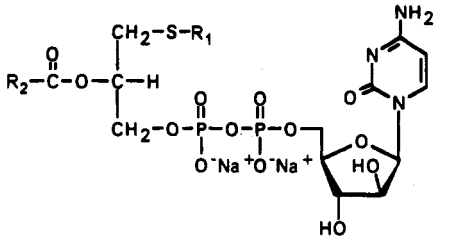
## Lipophilicity

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 II. $P$ values for the water-soluble conjugates ranged from 0.249 and 0.388, while those of the water-insoluble (**1, 2, 10**) were 0.543–0.616. The $P$ value for the slightly water-soluble conjugate **7** was 0.268. Thus, the increase in lipophilicity of the water-soluble conjugates was about 20-fold as compared to that of *ara*-C ($p = 0.013$), while that of the water-insoluble was more than 40-fold.

## Electron Microscopy

The ultrastructures of both sonicated and shaken solutions of conjugate **7** were observed by freeze-fracture electron microscopy as described previously.[16] The con-

**Table I.** *ara*-C Conjugates of Thioether Phospholipids



| compd | $R_1$ | $R_2$ | yield, % | mp, °C | formula | analysis |
|---|---|---|---|---|---|---|
| 3 | $C_{10}H_{21}$ | $C_{15}H_{31}$ | 20 | 183–188 | $C_{38}H_{69}N_3O_{13}SP_2 \cdot 2Na \cdot 2.75H_2O$ | C,H,N |
| 4 | $C_{12}H_{25}$ | $C_{13}H_{27}$ | 19 | 195–200 | $C_{38}H_{69}N_3O_{13}SP_2 \cdot 2Na \cdot 3H_2O$ | C,H,N |
| 5 | $C_{14}H_{29}$ | $C_{11}H_{23}$ | 16 | 220–230 | $C_{38}H_{69}N_3O_{13}SP_2 \cdot 2Na \cdot 4H_2O$ | C,H,N |
| 6 | $C_{14}H_{29}$ | $C_{13}H_{27}$ | 19 | 201–202 | $C_{40}H_{73}N_3O_{13}SP_2 \cdot 2Na \cdot H_2O$ | C,H,N |
| 7 | $C_{14}H_{29}$ | $C_{15}H_{31}$ | 42 | 202–204 | $C_{42}H_{77}N_3O_{13}SP_2 \cdot 2Na$ | C,H,N |
| 8 | $C_{16}H_{33}$ | $C_{11}H_{23}$ | 15 | 187–189 | $C_{40}H_{73}N_3O_{13}SP_2 \cdot 2Na \cdot 2.5H_2O$ | C,H,N |
| 9 | $C_{16}H_{33}$ | $C_{13}H_{27}$ | 27 | 195–198 | $C_{42}H_{77}N_3O_{13}SP_2 \cdot 2Na \cdot H_2O$ | C,H,N |
| 10 | $C_{16}H_{33}$ | $C_{17}H_{35}$ | 27 | 194–197 | $C_{46}H_{85}N_3O_{13}SP_2 \cdot 2Na$ | C,H,N |
| 11 | $C_{18}H_{37}$ | $C_{11}H_{23}$ | 15 | 188–191 | $C_{42}H_{77}N_3O_{13}SP_2 \cdot 2Na \cdot 2H_2O$ | C,H,N |
| 12 | $C_{18}H_{37}$ | $C_{13}H_{27}$ | 14 | 199–202 | $C_{44}H_{81}N_3O_{13}SP_2 \cdot 2Na \cdot H_2O$ | C,H,N |

jugate in sonicated solution existed as micellar disks with diameters varying from 0.01 to 0.09 μm when observed by electron microscopy after freeze-fracture (Figure 1A). The morphology of 7 in the shaken solution was not any different from that of the sonicated solution, and the micellar disk size was 0.01–0.15 μm in diameter (Figure 1B).

## Antitumor Activity

In Table III, conjugates 1–12 were compared for in vivo antitumor activity against ip-implanted L1210 lymphoid leukemia in DBA/2J mice according to the procedures outlined in the NCI protocols[17] with some modifications including inoculation of $1 \times 10^6$ cells as oposed to $1 \times 10^5$ cells and a 45-day observation period. A sonicated solution of the micelle-forming conjugates 7 and 10 at optimum single dose (400 mg/kg) produced significant antitumor activity (%ILS > 421%) with 50% long-term survivors (>45 days), which was comparable to those of the previous micelle-forming conjugate 1 (Cytoros). Micellar solution of 7 by shaking also gave a comparable antitumor activity. In contrast, the water-soluble conjugates 3–6, 8, 9, 11, and 12 at single doses (300–400 mg/kg) were found to be somewhat less effective (ILS 81–386% with 0–33% long-term survivors) than the micelle-forming conjugates. However, multiple doses of 3, 11, and 12 (150 mg/kg per day) given on days 1, 5, and 9 improved the efficacy (%ILS 200 to 464% with 0–50% long-term survivors). Particularly, treatment with multiple doses of conjugate 4 (200 mg/kg per day) given on days 1, 4, and 7 resulted in 67% long-term survivors. Table IV shows the effects of 7, 9, and appropriate controls (*ara*-C and Cytoros) against ic-implanted L1210 lymphoid leukemia in DBA/2J mice. Administration (ip) of a single dose (300 mg/kg) of 7 and 9 to the leukemic mice increased their life span by 192 and 130%, respectively, which was comparable to Cytoros. A single dose of *ara*-C was found to be ineffective.

## Discussion

The solubility studies indicate that the conjugates with *sn*-1 alkyl (<$C_{18}$) and *sn*-2 fatty acyl (<$C_{14}$) substituents of the thioglycerol are water-soluble and those with *sn*-1 alkyl (>$C_{14}$) and *sn*-2 fatty acyl (>$C_{16}$) are sparingly soluble (Table II). The latter form micelles in water since they are amphiphiles. Upon sonication, conjugates 7 and 10 exist as micellar disks (size 0.01–0.09 μm) which are similar to those formed from Cytoros (1) and 2.[9] Conjugate 7 formed a micellar solution both by sonication and shaking of the water suspension.

The micelle-forming conjugates exhibited a significant antitumor activity with a single-dose treatment of both ip- and ic-inoculated L1210 leukemic mice, while the water-soluble conjugates with a single dose produced somewhat less efficacy (Table III). However, the water-soluble conjugates with three divided doses gave comparable efficacy to the micelle forming with a single dose. These results indicate that micellization of the conjugates further improves their antitumor activity. In fact, administration of a micellar solution of Cytoros into L1210 leukemic mice gave a greater intracellular retention of *ara*-CTP than that resulting from *ara*-C.[9,13] Besides increased *ara*-CTP retention, other possible pharmacologically favorable properties of micellar solution of the conjugates are rapid interaction with serum lipoproteins,[18] 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.[19]

In summary, conjugate 7 and the water-soluble 4, 6, 9, 11, and 12 warrant further investigation because of their convenient formulation and significant antitumor activity.

**Table II.** 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 (Cytoros) | 34 | 0.2 | 0.583 | 273 (8.60) | 283 (12.07) | 273 (7.67)[c] |
| 2 | 32 | 0.2 | 0.540 | 273 (7.83) | 283 (11.64) | 273 (8.44)[c] |
| 3 | 26 | >58 | 0.317 | 272 (7.28) | 282 (11.46) | 273 (7.46) |
| 4 | 26 | >68 | 0.317 | 273 (6.65) | 283 (9.72) | 274 (7.66) |
| 5 | 26 | >50 | 0.339 | 273 (6.25) | 284 (7.76) | 274 (6.16) |
| 6 | 28 | >60 | 0.284 | 273 (6.37) | 284 (7.63) | 273 (6.76) |
| 7 | 30 | 1.5 | 0.268 | 274 (7.05) | 283 (8.61) | 274 (7.30) |
| 8 | 28 | >64 | 0.286 | 273 (6.67) | 284 (9.50) | 273 (7.08) |
| 9 | 30 | >53 | 0.249 | 274 (7.21) | 284 (9.33) | 274 (7.84) |
| 10 | 34 | 0.2 | 0.616 | 274 (6.80) | 284 (8.91) | 275 (5.92) |
| 11 | 30 | >54 | 0.337 | 273 (6.51) | 284 (7.36) | 273 (7.00) |
| 12 | 32 | >62 | 0.388 | 274 (6.42) | 284 (9.43) | 274 (6.05) |

[a] Determined by UV absorption at 273 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 6.

**Table III.** 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] | survival days % ILS[d] | 45 day survivors |
|---|---|---|---|---|---|---|---|
| 1 (Cytoros) | 1 | 300 (292) | m | 21 to >45 | >45.0/7.0 | >543 | 4/6 |
| | 1, 5, 9 | 150 (146) | m | 13 to >45 | 27.5/7.0 | 293 | 1/6 |
| 2 | 1 | 300 (300) | m | 12 to >45 | >37.5/7.0 | >436 | 2/6 |
| 3 | 1 | 400 (437) | s | 14–16 | 15.0/7.0 | 114 | 0/6 |
| | 1, 5, 9 | 150 (164) | s | 17–28 | 21.0/7.0 | 200 | 0/6 |
| 4 | 1 | 300 (328) | s | 2–17 | 14.5/8.0 | 81 | 0/6 |
| | 1, 4, 7 | 200 (218) | s | 14 to >45 | >45.0/8.0 | >463 | 4/6 |
| 5 | 1 | 400 (437) | s | 15–19 | 16.0/7.0 | 129 | 0/6 |
| 6 | 1 | 300 (318) | s | 15 to >45 | 24.0/7.0 | 243 | 2/6 |
| 7 | 1 | 400 (411) | m | 19 to >45 | >36.5/7.0 | >421 | 3/6 |
| | | 400 (411) | ms | 14 to >45 | 34.5/7.0 | 393 | 1/6 |
| | 1, 5, 9 | 150 (154) | m | 27 to >45 | 29.5/7.0 | 321 | 2/6 |
| 8 | 1 | 300 (318) | s | 17 to >45 | 19.5/7.0 | 178 | 2/6 |
| 9 | 1 | 300 (309) | s | 29 to >45 | 34.0/7.0 | 386 | 2/6 |
| 10 | 1 | 400 (389) | m | 20 to >45 | >43.5/7.0 | >521 | 3/6 |
| 11 | 1 | 300 (309) | s | 17 to >45 | 25.0/8.0 | 213 | 1/6 |
| | 1, 5, 9 | 150 (154) | s | 29 to >45 | 33.0/7.0 | 371 | 2/6 |
| 12 | 1 | 400 (400) | s | 9 to >45 | 25.0/8.0 | 213 | 1/6 |
| | 1, 5, 9 | 150 (150) | s | 30 to >45 | >39.5/7.0 | >464 | 3/6 |

[a] Each group of five to eight DBA/2J mice (male, 20–29 g) received ip inoculation of $1 \times 10^6$ cells on day 0. Treatments (ip) were initiated on day 1. [b] m, micelles by sonication; s, solution; and ms, micelles by shaking (see text for detail). [c] Calculated based on survivors accoridng to the NCI protocols.[17] [d] Increase in life span: (T/C − 1) × 100.

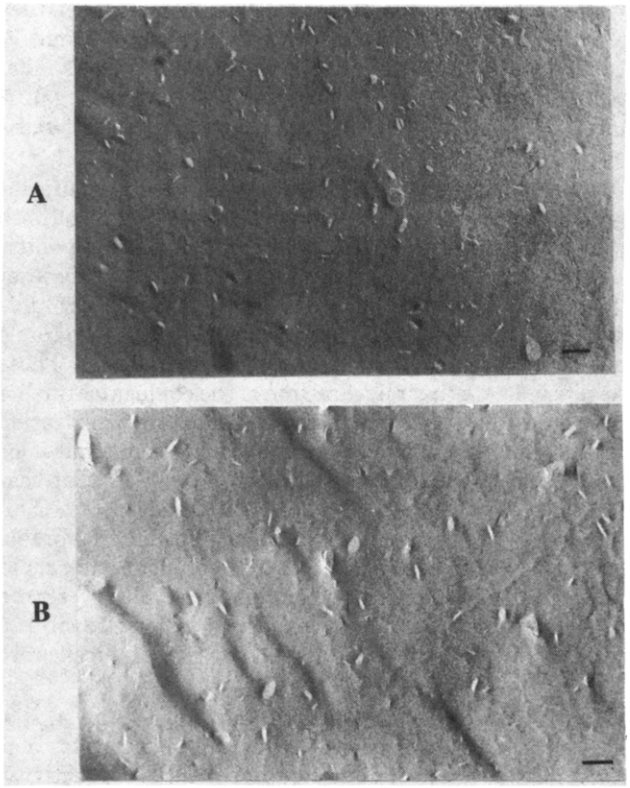



**Figure 1.** Freeze-fracture electron micrographs of conjugate 7 in micellar form by sonication (A) and shaking (B). Bars, 0.1 μm.

## 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. Mass spectra were obtained using a Finnigan MAT 90 spectrometer with negative-ion FAB ionization. AG1-X8 (Bio-Rad), [(diethylamino)ethyl]cellulose (DE-52, Whatman), and CG-50 (Sigma) 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.[20] 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,[21] *ara*-CMP morpholidate (**19**),[14] and *rac*-1-*S*-alkyl-1-thioglycerols (**13**)[15] were prepared by a literature procedure.

***rac*-1-*S*-Tetradecyl-1-thioglycerol (13, $R_1 = C_{14}H_{29}$).** To a mixture of 3-mercapto-1,2-propanediol (DL-1-thioglycerol) (64.9 g, 0.6 mol) in 300 mL of MeOH and 1-bromotetradecane (83.2 g, 0.3 mol) in 600 mL of hexanes was added dropwise 780 mL of 1 N KOH in MeOH at room temperature for a period of 1 h, and then the mixture was stirred at room temperature for 2 days. The white solid was filtered and washed with MeOH, 50% aqueous MeOH, and MeOH (300 mL each). The additional product was obtained by concentrating the filtrate to turbidity: total yield 89.5 g (98%); mp 59–61 °C; $^1$H NMR ($CDCl_3$) δ 0.85 (3, t, $J$ = 6 Hz, $CH_3$), 1.27–1.63 (24, m, $(CH_2)_{12}$), 2.50 (2, t, $J$ = 6 Hz, $CH_2SCH_2$), 2.60 (2, d, $J$ = 6 Hz, $CH_2SCH_2$), 3.40–3.80 (3, m, 1-$CH_2$, 2-$CH$). Anal. ($C_{17}H_{36}O_2S \cdot H_2O$) C, H.

Other *rac*-1-*S*-alkyl-1-thioglycerols (**13**) were prepared from 3-mercapto-1,2-propanediol in an analogous manner: yield >95%.

***rac*-1-*S*-Tetradecyl-3-*O*-(*tert*-butyldimethylsilyl)-1-thioglycerol (14, $R_1 = C_{14}H_{29}$).** A mixture of **13** ($R_1 = C_{14}H_{29}$) (72.7 g, 0.24 mol), *tert*-butyldimethylsilyl chloride (39.2 g, 0.26 mol), imidazole (35.4 g, 0.52 mol), and DMF (500 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 86 g (88.1%) and was used for the next step without further purification.

Other *rac*-1-*S*-alkyl-3-*O*-(*tert*-butyldimethylsilyl)-1-thioglycerols (**14**) were prepared by using an analogous procedure: yield >85%.

***rac*-1-*S*-Tetradecyl-2-*O*-palmitoyl-3-*O*-(*tert*-butyldimethylsilyl)-1-thioglycerol (15, $R_1 = C_{14}H_{29}$ and $R_2 = C_{15}H_{31}$).** To a mixture of the above product (86 g, 0.21 mol), anhydrous pyridine (20 mL), and toluene (50 mL) was added dropwise palmitoyl chloride (60.5 g, 0.22 mol) at room temperature, and the mixture was stirred at room temperature for 1 day. The

Table IV. Antitumor Activity against Ic-Implanted L1210 Lymphoid Leukemia in Mice[a]

| compd | treatment schedule, qd | dose, mg (μmol)/kg per day | wt change (g/mouse) on day 8 | survival days | | | 45 day survivors |
|---|---|---|---|---|---|---|---|
| | | | | range | median T/C[b] | % ILS[c] | |
| *ara*-C | 1 | 71 (292) | −3.8 | 7–8 | 8.0/6.5 | 23 | 0/6 |
| | 1–5 | 100 (411) | −3.1 | 15–18 | 16.0/7.0 | 129 | 0/6 |
| | 1–9 | 200 (822) | −6.1 | 11–14 | 14.0/7.0 | 100 | 0/6 |
| 1 (Cytoros) | 1 | 300 (292) | −2.4 | 14–20 | 19.0/6.5 | 192 | 0/6 |
| 7 | 1 | 300 (309) | +0.5 | 12–22 | 19.0/6.5 | 192 | 0/6 |
| 9 | 1 | 300 (309) | −1.8 | 9 to >45 | 15.0/6.5 | 130 | 1/6 |

[a] Each group of six DBA/2J mice (male, 20–29 g) received ic inoculation of $1 \times 10^5$ cells on day 0. Treatments (ip) were initiated on day 1. [b] Calculated based on survivors according to the NCI protocols.[17] [c] Increase in life span: (T/C − 1) × 100.

mixture was then partitioned between $Et_2O$ and $H_2O$ (500 mL each). The organic layer was washed with 0.5 N $H_2SO_4$, saturated $NaHCO_3$, and $H_2O$ (200 mL each) and then evaporated to dryness. The residue was crystallized from 95% EtOH at 0–5 °C. The solid was filtered and washed with 95% EtOH. The additional product was obtained by concentrating the filtrate to turbidity: total yield, 125 g (96%). The soft solid was essentially homogeneous by TLC and used for the next step without further purification.

Other *rac*-1-*S*-alkyl-2-*O*-acyl-3-*O*-(*tert*-butyldimethylsilyl)-1-thioglycerols (**15**) were prepared in an analogous manner: yield >95%.

***rac*-1-*S*-Tetradecyl-2-*O*-palmitoyl-1-thioglycerol (16, $R_1$ = $C_{14}H_{29}$ and $R_2$ = $C_{15}H_{31}$).** To a mixture of above product (124 g, 0.19 mol) in HOAc (24 mL) and THF (500 mL) was added dropwise 1 M tetrabutylammonium fluoride in THF (242 mL) for a period of 2 h at 5–10 °C and the mixture was stirred at room temperature for 6 h. After cooling at 0–5 °C overnight, the solid was filtered and washed with ice-cold 95% EtOH. The filtrate was evaporated to dryness, and the residue was treated with ice-cold 95% EtOH. The combined solids were dissolved in boiling 95% EtOH, and the solution was cooled to room temperature overnight, which resulted in an isomer, *rac*-1-*S*-tetradecyl-3-*O*-palmitoyl-1-thioglycerol (**17**, $R_1 = C_{14}H_{29}$ and $R_2 = C_{15}H_{31}$). After filtration, the filtrate was cooled to 0–5 °C overnight, and the white product was filtered and washed with cold 95% EtOH. Repeated recrystallizations of the crude products in this manner gave 41 g (39.7% yield): mp 40–42 °C; $^1$H NMR δ 0.87 (6, t, $J$ = 6 Hz, 2 $CH_3$), 1.27–1.67 (50, m, $(CH_2)_{12}$, $(CH_2)_{13}$), 2.30 (2, t, $J$ = 7.5 Hz, $CH_2CH_2CO$), 2.47 (2, t, $J$ = 6 Hz, $CH_2SCH_2$), 2.70 (2, d, $J$ = 6 Hz, $CH_2SCH_2$), 3.77 (2, d, $J$ = 6 Hz, 3-$CH_2$), 4.96 (1, m, 2-$CH$). Anal. ($C_{33}H_{66}O_3S$·0.5$H_2O$) C, H.

Other *rac*-1-*S*-alkyl-2-*O*-acyl-1-thioglycerols (**16**) were prepared in analogous manner: yield 52–71%.

***rac*-1-*S*-Tetradecyl-2-*O*-palmitoyl-1-thioglycerol 3-Phosphate (18, $R_1 = C_{14}H_{29}$ and $R_2 = C_{15}H_{31}$).** To an ice-cold mixture of $POCl_3$ (22.8 g, 0.16 mol) and hexanes (50 mL) was added dropwise triethylamine (16.2 g, 0.16 mol) in hexanes (50 mL). To this mixture was added dropwise a solution of the above product (62.0 g, 0.11 mol) in toluene (500 mL) at 0–5 °C over a period of 1.5 h, and the mixture was stirred at room temperature overnight. Water (50 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 $H_2O$ (250 mL). The organic layer was dried over $Na_2SO_4$ and evaporated to dryness. The residue was crystallized from hexanes at −10 °C and then recrystallized from $Et_2O$ at −10 °C: yield 26.8 g (39%); mp 75–77 °C; $^1$H NMR δ 0.85 (6, t, 2 $CH_3$), 1.27–1.73 (50, m, $(CH_2)_{12}$, $(CH_2)_{13}$), 2.13–2.67 (6, m, $CH_2CH_2CO$, $CH_2SCH_2$), 4.12 (2, m, 3-$CH_2$), 5.07 (1, m, 2-$CH$). Anal. ($C_{33}H_{67}O_6SP$·1.5$H_2O$) C, H.

*rac*-1-*S*-Hexadecyl-2-*O*-stearoyl-1-thioglycerol 3-phosphate (**18**, $R_1 = C_{16}H_{33}$ and $R_2 = C_{17}H_{35}$) was prepared in an analogous manner: yield 29.1%; mp 75–80 °C soften. Anal. ($C_{37}H_{75}O_6SP$·1.5$H_2O$) C, H.

Other *rac*-1-*S*-alkyl-2-*O*-acyl-1-thioglycerol 3-phosphates (**18**) were also prepared in an analogous manner, and the crude phosphates were used for the condensation.

***ara*-CDP-*rac*-1-*S*-tetradecyl-2-*O*-palmitoyl-1-thioglycerol (7).** The above phosphate (20 g, 32 mmol) was dried azeotropically with pyridine twice and mixed with *ara*-CMP morpholidate (**19**) (17.8 g, 26 mmol) followed by coevaporation with pyridine three times. The dried mixture was then mixed with anhydrous pyridine (800 mL) and stirred at room temperature for 7 days. The solvent was evaporated to dryness, and the residue was coevaporated with toluene to remove the residual pyridine. The residue was dissolved in 1 L of $CHCl_3$–MeOH–$H_2O$ (2:3:1) and then mixed with 0.5 N HCl (100 mL). The organic layer was separated, and the aqueous layer was extracted with $CHCl_3$ (2 × 200 mL). The combined organic layers were evaporated to dryness, and the residue was dissolved in 1 L of $CHCl_3$–MeOH–$H_2O$ (2:3:1). The solution was applied to a DE-52 ($AcO^-$) column (10 × 30 cm) equilibrated with the solvent. The column was eluted with $CHCl_3$–MeOH–$H_2O$ (2:3:1) (2.5 L) and then with 0.04 M $NH_4OAc$ in the same solvent. The 0.04 M $NH_4OAc$ fractions between 10 and 17 L were evaporated to a small volume, and the solid was collected on a filter followed by washing with 50% aqueous $Me_2CO$ and then $Me_2CO$. The solid ($NH_4$ salt of **7**) was dissolved in $CHCl_3$–MeOH–$H_2O$ (2:3:1), and the solution was passed through a CG-50 ($Na^+$) column (2.5 × 15 cm). The column was washed further with the same solvent until no UV-absorbing material was detected. The combined eluate was cooled at 0–5 °C overnight, and the solid was filtered off. The filtrate was evaporated to a small volume, and the product (Na salt) was filtered, washed with acetone, and dried in vacuo: yield 10.53 g (42%); mp 202–204 °C; $^1$H NMR ($CDCl_3$–$CD_3OD$–$D_2O$, 2:3:1) δ 0.83 (6, t, $J$ = 6 Hz, 2 $CH_3$), 1.27–1.73 (50, m, $(CH_2)_{12}$, $(CH_2)_{13}$), 2.32 (2, t, $J$ = 7 Hz, $CH_2CH_2CO$), 2.50 (2, t, $J$ = 7 Hz, $CH_2SCH_2$), 2.76 (2, t, $J$ = 7 Hz, 1-$CH_2$), 3.97–4.73 (7, m, 3-$CH_2$, $H$-2′, $H$-3′, $H$-4′ $H$-5′), 5.07 (1, m, 2-$CH$), 5.92 (1, d, $J$ = 7.5 Hz, cytosine $H$-5), 6.08 (1, d, $J$ = 5 Hz, $H$-1′), 7.80 (1, d, $J$ = 7.5 Hz, cytosine $H$-6); MS $m/z$ (FAB) 962.3 (M − H)$^-$ for free acid.

The conjugates **3–6** and **8–12** in Table I were prepared in an analogous manner.

**Water Solubility.** Conjugate (50 mg) in 1 or 5 mL of sterile water for injection, USP, was shaken at room temperature using a Thomas Clinical Rotator at speed 1 for 2 h, and the solution or suspension was filtered through a membrane filter (0.22 μm). Concentration of the free conjugate in the filtrate was checked by quantitative UV at 273 nm.

**Partition Coefficient Measurements.** 1-Octanol/aqueous-phase partition coefficients were determined at room temperature using the shake-flask procedure described previously.[22] 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.

**Freeze-Fracture Electron Microscopy.** Samples were frozen without cryoprotectants, using a home-built quick-freeze apparatus described previously.[16] Representative micrographs were taken using a Hitachi Model H-600 electron microscope.[9]

**Biological Studies. Antitumor Activity in Vivo.** DBA/2J male mice in groups of 6 (wt 20–29 g) were inoculated ip with $1 \times 10^6$ (or ic with $1 \times 10^5$) L1210 lymphoid leukemia cells,[17] and an aqueous or a sonicated solution of the conjugates was given ip as reported earlier.[9] Each drug was tested over a wide range of doses. The results from the optimal dose levels are shown in Tables III and IV.

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## References


(1) This material was presented in part in May 1990 at the 81st Annual meeting of the American Association for Cancer Research in Washington, DC (Abstract 2492).

(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; 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; *ara*-CDP-DL-dipalmitin, *ara*-CDP-L-1,2-dipalmitin.

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