Nucleoside Conjugates. 11. Synthesis and Antitumor Activity of 1-β-D-Arabinofuranosylcytosine and Cytidine Conjugates of Thioether Lipids[1]

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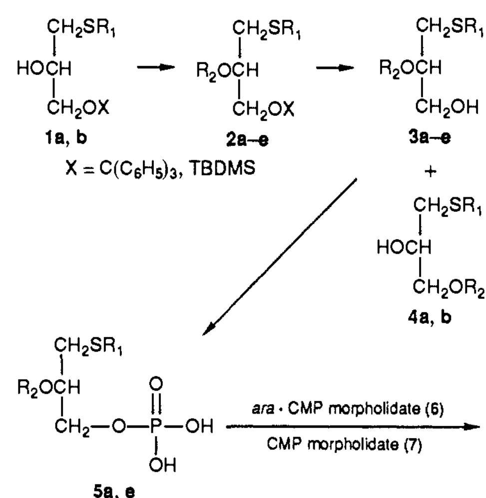
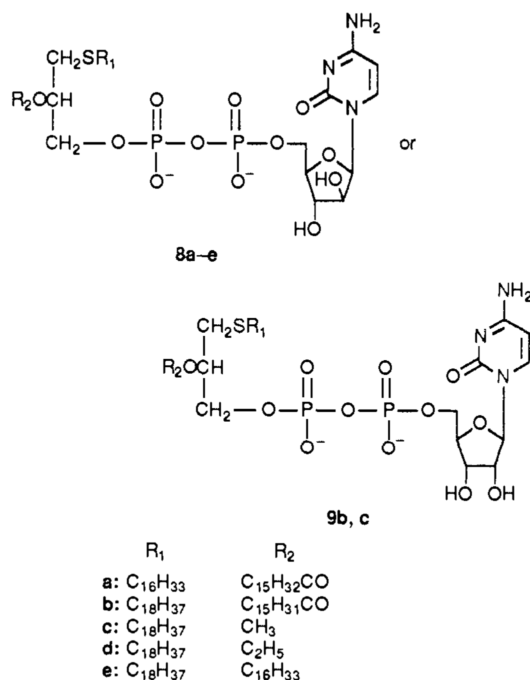
Five 1-β-D-arabinofuranosylcytosine conjugates and two cytidine conjugates of thioether lipids (1-*S*-alkylthioglycerols) linked by a pyrophosphate diester bond have been prepared and their antitumor activity against an *ara*-C[2] sensitive (L1210/0) and two *ara*-C resistant L1210 lymphoid leukemia sublines in mice were evaluated. These prodrugs of *ara*-C include *ara*-CDP-*rac*-1-*S*-hexadecyl-2-*O*-palmitoyl-1-thioglycerol (**8a**), *ara*-CDP-*rac*-1-*S*-octadecyl-2-*O*-palmitoylthioglycerol (**8b**), and *ara*-CDP-rac-1-*S*-octadecyl-2-*O*-methyl(or -ethyl, -hexadecyl)thioglycerols (**8c–e**). The cytidine conjugates include CDP-*rac*-1-*S*-octadecyl-2-*O*-palmitoyl(or -methyl)-1-thioglycerols (**9a** and **9b**). Sonicated solutions of the conjugates existed in the form of micellar disks (size 0.01–0.04 μm). Single doses (200–400 mg/kg) of **8a** and **8b** produced significant increase in life span (257–371%) in mice bearing ip implanted L1210/0 leukemia. In contrast, conjugates **8c–e** were less effective (ILS 19–75%) and cytidine conjugates (**9a** and **9b**) were ineffective. Even though **8a** and **8b** were found to be curative in a high percentage of mice bearing ip implanted partially *ara*-C resistant L1210 subline [L1210/*ara*-C(I)], they were completely ineffective against deoxycytidine kinase deficient *ara*-C resistant L1210 subline [L1210/*ara*-C(II)]. However, the present results, together with the previous, demonstrate that **8a** and **8b** are promising new prodrugs of *ara*-C with improved efficacy.

Ether lipid and thioether lipid analogues of the naturally occurring 1-octadecyl-2-acetyl-*sn*-glycero-phosphocholine (platelet-activating factor) have demonstrated antineoplastic properties in vitro and in vivo[3,4] and first clinical phase I and II trials with their synthetic analogues such as ET-18-OCH_3 and BM 41.440 are in progress.[5,6] These interesting biological activities, together with favorable activity of the *ara*-C conjugates of 1-*O*-acylphospholipids such as *ara*-CDP-L-dipalmitin[7-9] led us to synthesize a series of *ara*-C conjugates of 1-*O*-alkyl- (ether) and 1-*S*-alkyl- (thioether) phospholipids.[10-12] The rationale is that the conjugates are not only new prodrugs of *ara*-C but also may generate two cytotoxic groups with different target sites, nucleic acid synthesis and membrane, inside a neoplastic cell. Most of them displayed a significant antitumor activity against L1210 and P388 leukemia in mice.[10,12] Particularly, the *ara*-C conjugate of a thioether lipid, *ara*-CDP-*rac*-1-*O*-octadecyl-2-*O*-palmitoyl-1-thioglycerol (**8b**) displayed a significant therapeutic activity in mice with colon 26 carcinoma, M5076 sarcoma, C-1300 neuroblastoma, and 3-Lewis lung carcinoma.[13-16] Further-

Scheme I





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(2) The abbreviations used are: *ara*-C, 1-β-D-arabinofuranosylcytosine; *ara*-CDP, 1-β-D-arabinofuranosylcytosine 5′-diphosphate; ET-18-OCH_3, 1-*O*-octadecyl-2-*O*-methyl-*rac*-glycero-3-phosphocholine; BM 41.440, 1-hexadecylmercapto-2-methoxymethyl-*rac*-glycero-3-phosphocholine; *ara*-CDP-L-dipalmitin, *ara*-CDP-L-1,2-dipalmitin; TBDMS, *tert*-butyldimethylsilyl; ILS, increase in life span.

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Table I. Thioether Lipids

$$\begin{array}{c} CH_2SR_1 \\ | \\ R_2OCH \\ | \\ CH_2OR_3 \end{array}$$

compd	R_1	R_2	R_3	method	mp, °C	% yield	formula	^{1}H NMR data[b] 2-CH
3a	$C_{16}H_{33}$	$C_{15}H_{31}CO$	H	A	42–43	60	$C_{35}H_{70}O_3S$	4.97 (quintet)
				B		15		
3b	$C_{18}H_{37}$	$C_{15}H_{31}CO$	H	A	43–44	63	$C_{37}H_{74}O_3S$	4.98 (quintet)
3c	$C_{18}H_{37}$	CH_3	H	A	43–44	76	$C_{22}H_{46}O_2S$	3.43 (m)
				B		95		
3d	$C_{18}H_{37}$	C_2H_5	H	A	48–50	45	$C_{23}H_{48}O_2S$	3.58 (m)
				B		95		
3e	$C_{18}H_{37}$	$C_{16}H_{33}$	H	A	40–42	43	$C_{37}H_{76}O_2S$	3.50 (m)
4a	$C_{16}H_{33}$	H	$C_{15}H_{31}CO$	A	66–67	30	$C_{35}H_{70}O_3S$	3.88 (m)
				B		81		
4b	$C_{18}H_{37}$	H	$C_{15}H_{31}CO$	A	67–68	29	$C_{37}H_{74}O_3S$	3.87 (m)
5a	$C_{16}H_{33}$	$C_{15}H_{31}CO$	$P(O)(OH)_2$	C	68–70	56	$C_{35}H_{71}O_6SP$	5.10 (m)
5b	$C_{18}H_{37}$	$C_{15}H_{31}CO$	$P(O)(OH)_2$	C	65–67	45	$C_{37}H_{75}O_6SP$	5.12 (m)
5c	$C_{18}H_{37}$	CH_3	$P(O)(OH)_2$	C	58–60	52	$C_{22}H_{47}O_5SP$	3.45 (m)
5d	$C_{18}H_{37}$	C_2H_5	$P(O)(OH)_2$	C	56–58	56	$C_{23}H_{49}O_5SP$	3.63 (m)
5e	$C_{18}H_{37}$	$C_{16}H_{33}$	$P(O)(OH)_2$	C	68–70	60	$C_{37}H_{77}O_5SP$	3.53 (m)

[a] Analyses for C and H for **3a–e** and **4a,b** and for C, H, S, and P for **5b**. Compounds **5a,c–e** were homogeneous by TLC and used for the condensation without elemental analyses. [b] All shifts of the *sn*-2-methine proton measured in δ from Me_4Si.

more, this compound showed inhibition of liver metastases of M5076 sarcoma[13] and lung metastases of 3-Lewis lung carcinoma in mice.[16]

In order to study further structure–activity relationships of the conjugates of this type, a series of the conjugates (**8c–e**) with different alkyl chains at the *sn*-2-position of the glycerol moiety and cytidine analogues **9b** and **9c** have been synthesized. As an extension of our previous works, this paper describes the detailed synthetic procedures for new 1-*S*-alkyl analogues of *ara*-CDP-L-dipalmitin and their antitumor effects against L1210 lymphoid leukemia in mice.

Chemistry

The synthetic route used to prepare *ara*-C conjugates **8a–e** and cytidine conjugates **9b** and **9c** is shown in Scheme I. A major task of the synthetic route was synthesis of pure 1-*S*-alkylphosphatidic acids **5a–e**. *rac*-1-*S*-Hexadecyl-1-thioglycerol (DL-thiochimyl alcohol) and *rac*-1-*S*-octadecyl-1-thioglycerol (DL-thiobatyl alcohol) were prepared by alkylation of the mercaptan of DL-1-thioglycerol with hexadecyl or octadecyl bromide and alcoholic potassium hydroxide.[17] The primary alcohol of the 1-*S*-alkyl thioglycerols was then protected with either trityl chloride[18,19] or *tert*-butyldimethylsilyl chloride in the presence of imidazole and DMF.[20] The protected alkylthioglycerols **1a** and **1b** were then acylated with palmitoyl chloride and pyridine or alkylated with CH_3I, C_2H_5I, or hexadecyl bromide and NaH. The resulting compounds **2a–e** were purified by crystallization from a large amount of boiling 95% EtOH. The trityl group was removed by treatment of **2c–e** in CH_2Cl_2 with BF_3–MeOH at 0 °C,[21] and 1-*S*-alkyl-2-*O*-alkyl-1-thioglycerols **3c** and **3d** were obtained in good yield (95%, Table I). However, the deblocking of the trityl group of the 2-*O*-palmitoylthioglycerols (**2a** and **2b**, X = trityl) by this method resulted in a substantial acyl migration and low yields (15%) of the desired products **3a** and **3b**. Thus, the TBDMS protected 1-*S*-alkylthioglycerols (**1a** and **1b**, X = TBDMS) were used for the preparation of the 2-*O*-palmitoylthioglycerols (**2a** and **2b**, X = TBDMS). The TBDMS group was removed by treatment of **2a** and **2b** in HOAc with tetrabutylammonium fluoride in THF at 5–10 °C first and then at room temperature.[20] The desired **3a** and **3b** were obtained in 60–63% yield in spite of the continuous acyl migration during the repeated crystallization in 95% EtOH. The thermodynamically more stable isomers 1-*S*-alkyl-3-*O*-palmitoyl-1-thioglycerols **4a** and **4b** were obtained also in 30% yield. Structure assignments of **3a** and **3b** were confirmed by ^{1}H NMR spectrometry. The *sn*-2 methine proton of **3a** and **3b** gave a first-order quintet at 4.98 ppm, whereas it was a second-order multiplet at 3.40–3.88 ppm in **4a**, **4b**, and 1-*S*-alkylglycerols. These values are in good agreement with those for the 1-*O*-alkylglycerol derivatives[10] and isomeric lysophophatidylcholines reported earlier.[22]

Compounds **3a–e** were phosphorylated with $POCl_3$ and Et_3N at 0–5 °C as outlined previously[10] and the resulting compound **5a–e** were purified by successive crystallization from hexanes and Et_2O, and the final yield was 45–60% (Table I). Condensation of **5a–e** with *ara*-CMP morpholidate[23] (**6**) in pyridine gave conjugates **8a–e** in overall yields of 15–38% (Table II). Likewise, condensation of **5b** and **5c** with cytidine 5′-phosphoromorpholidate[24] (**7**) gave conjugates **9b** and **9c** in 29–31% (Table II). Struc-

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Table II. *ara*-C and Cytidine Conjugates of Thioether Lipids

					^{1}H NMR data[c]			
					glycerol:	cytosine		sugar:
compd	mp, °C	% yield	formula	UV_{max},[b] nm ($\epsilon \times 10^{-3}$)	2-CH	H-5	H-6	H-1′
8a	199–200	25	$C_{44}H_{81}N_3O_{13}SP_2\cdot 2Na$	(i) 273 (7.83) (ii) 283 (11.64) (iii) 273 (8.44)	5.05	5.91	7.78	6.08
8b	206–207	38	$C_{46}H_{85}N_3O_{13}SP_2\cdot 2Na\cdot 2H_2O$	(i) 273 (8.60) (ii) 283 (12.07) (iii) 273 (7.67)	5.08	5.94	7.84	6.11
8c	182–184	15	$C_{31}H_{57}N_3O_{12}SP_2\cdot 2Na$	(i) 273 (9.29) (ii) 283 (13.22) (iii) 273 (8.52)	3.42	6.00	7.97	6.07
8d	216–218	32	$C_{32}H_{59}N_3O_{12}SP_2\cdot 2Na\cdot H_2O$	(i) 273 (6.47) (ii) 283 (11.68) (iii) 273 (6.46)	3.63	6.00	7.88	6.18
8e	199–200	29	$C_{46}H_{87}N_3O_{12}SP_2\cdot 2Na\cdot 2H_2O$	(i) 273 (7.97) (ii) 283 (10.08) (iii) 273 (6.96)	3.63	5.97	7.83	6.13
9b	188–190	29	$C_{46}H_{85}N_3O_{13}SP_2\cdot 2Na\cdot 2H_2O$[d]	(i) 273 (8.68) (ii) 283 (11.78) (iii) 273 (8.23)	5.07	6.00	8.03	5.85
9c	185–187	31	$C_{31}H_{57}N_3O_{12}SP_2\cdot 2Na$[e]	(i) 273 (6.48) (ii) 283 (10.09) (ii) 273 (6.51)	3.53	6.02	8.02	5.82

[a] Analyses for C, H, S, and P for all compounds listed. [b] (i) $CHCl_3$–MeOH–H_2O (2:3:1), (ii) $CHCl_3$–MeOH–0.6 N HCl (2:3:1), and (iii) $CHCl_3$–MeOH–0.6 N NaOH (2:3:1). [c] All shifts of the glycerol *sn*-2-methine (m), cytosine H-5 (d, J = 7.5 Hz) and H-6 (d, J = 7.5 Hz), and the anomeric proton (d, J = 5.25 Hz) measured in δ from Me_4Si. [d] S: calcd, 2.99; found, 3.62. [e] H: calcd, 7.15; found 7.68. S: calcd, 3.99; found, 4.58.

Table III. Antitumor Activity against Ip Implanted L1210/0 Lymphoid Leukemia in Mice[a]

			survival days			
compd	treatment schedule, qd	dose, mg (μmol)/kg per day	range	median T/C[b]	% ILS[c]	45-day survivors
ara-C	1	400 (1644)	8–10	8.0/7.0	14	0
	1–5	200 (822)	15–17	16.0/7.0	129	0
	5	400 (1644)	10	10.0/7.0	43	0
8a	1	300 (300)	18–30	25.0/7.0	257	0
		400 (400)	16–>45	27.5/7.0	293	1
	5	300 (300)	15–21	17.0/8.0	113	0
		400 (400)	15–19	16.5/8.0	106	0
	1–5	60 (60)	16–37	26.5/8.0	231	0
		100 (100)	14–>45	36.5/8.0	356	2
8b	−1	400 (389)	12–>45	21.5/7.0	207	2
	0	400 (389)	14–>45	>42.5/7.0	>507[d]	3
	1	200 (195)	24–>45	32.0/7.0	357	1
		300 (292)	18–>45	33.0/7.0	371	1
		400 (389)	9–>45	29.5/7.0	321	1
	2	400 (389)	12–26	19.5/7.0	179	0
	5	200 (195)	15–16	15.0/7.0	114	0
		400 (389)	12–16	14.5/7.0	107	0
	1, 5, 9	100 (97)	18–29	25.0/8.0	213	0
	1, 15	200 (195)	22–32	27.5/7.0	293	0
		300 (292)	21–>45	24.5/7.0	243	1
	1, 8, 15, 22	100 (97)	16–32	24.0/7.0	243	0
		200 (195)	14–>45	24.5/7.0	250	1
	1–5	60 (58)	27–>45	31.0/8.0	288	1
8c	1	100 (124)	4–11	9.5/7.0	36	0
		200 (249)	6	6.0/7.0	−14	0
8d	1	100 (122)	9–10	9.5/8.0	19	0
		200 (244)	4–12	7.5/8.0	−6	0
8e	1	400 (395)	11–15	14.0/8.0	75	0
	1–5	80 (79)	14–19	16.0/8.0	100	0
		100 (99)	15–32	21.5/8.0	169	0
9b	1	300 (292)	7–9	7.5/8.0	−6	0
9c	1	200 (259)	4–8	8.0/7.5	7	0

[a] Each group of DBA/2J mice (wt 20–29 g) received ip inoculation of 1×10^6 cells on day 0. Treatments (ip) were initiated on the designated days. [b] Calculated based on survivors according to the NCI protocols.[26] [c] Increase in life span: $(T/C - 1) \times 100$. [d] As of day 45 (final day of observation).

tures were verified by elemental analysis, ^{1}H NMR, and UV spectrometry.

Antitumor Activity

Conjugates **8a–e** and **9b,c** were evaluated for in vivo antitumor activity against three L1210 leukemia cell lines which included *ara*-C sensitive (L1210/0) and two *ara*-C resistant [L1210/*ara*-C(I) and L1210/*ara*-C(II)] strains.[25] Preliminary antitumor activity of conjugates **8a,b,e** against L1210/0 lymphoid leukemia in mice have been reported

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

cell line	compd	treatment schedule qd	active dose range,[b] mg (μmol)/kg per day	optimum dose,[c] mg (μmol)/kg per day	survival days: range	survival days: median T/C[d]	survival days: % ILS	45-day survivors
L1210/ara-C (I)[f]	**8a**	1	200 (200)–400 (400)	300 (300)	>45	>45.0/9.0	>400[h]	6
		1, 5, 9	100 (100)–167 (166)	167 (166)	7->45	41.0/10.0	310	1
		1–5	40 (40)–80 (80)	60 (60)	29->45	>41.0/9.0	>356[h]	3
	8b	1	200 (195)–500 (486)	400 (389)	>45	>45.0/10.0	>350[h]	6
		1, 5, 9	100 (97)–167 (162)	133 (129)	>45	>45.0/10.0	>350[h]	6
		1–5	40 (39)–80 (78)	80 (78)	25->45	>45.0/10.0	>350[h]	4
L1210/ara-C (II)[g]	**8b**	1		300 (292)	8–10	9.0/9.0	0	0
	9b	1		300 (292)	7–8	8.0/9.0	–11	0
	9c	1		300 (373)	8–9	8.0/9.0	–11	0

[a] Each group of six DBA/2J mice (wt 20–26 g) received ip inoculation of 1×10^5 cells on day 0. Treatments (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 ≥50% over the controls. [c] Dose producing greatest increase in life span. [d] Calculated based on survivors according to the NCI protocols.[26] [e] Increase in life span: (T/C – 1) × 100. [f] Partially resistant to *ara*-C due to lower *ara*-C uptake than that of L1210/0.[25] [g] Completely resistant to *ara*-C due to deoxycytidine kinase deficiency. [h] As of day 45 (final day of observation).

previously.[12] Table III shows further antitumor effects of the conjugates on ip-implanted L1210/0 lymphoid leukemia in DBA/2J mice with different dose levels and treatment schedules according to the procedure outlined in the NCI protocol[26] with some modifications.[12] Previously, the single (400 mg/kg) and the multiple courses (200 mg/kg per day × 5) of therapy with *ara*-C produced ILS values of 14 and 129%, respectively.[12] In contrast, single doses (200–400 mg/kg) of **8a** and **8b**, which contained an acyl (palmitoyl) group at the *sn*-2-position of the glycerol moiety, produced a significant antitumor activity with increases in life span of 257–371% and one long-term survivor out of six animals. Multiple courses of drug therapy resulted in no gain in animal life span. The *ara*-C conjugates with a short alkyl (methyl or ethyl) group at the *sn*-2-position (**8c** and **8d**) were found to be toxic and did not improve the efficacy. However, the five consecutive doses (100 mg/kg per day × 5) of **8e**, which contained a longer alkyl (hexadecyl) group at the sn-2-position product an ILS value of 169%. The cytidine conjugates (**9b** and **9c**) were found to be ineffective. Conjugates **8a** and **8b** were also effective on survivals of the advanced state of L1210 leukemic mice. Single doses (200–400 mg/kg) of **8a** and **8b** on day 5 produced ILS values of 106–114%, while that of *ara*-C with a single dose (400 mg/kg) on day 5 was 43%.

In order to determine whether the conjugates acted as a sustained release form, a single dose (400 mg/kg) of **8b** was administered ip on day -1, 0, 1, or 2, and the life-prolonging effects were observed (Table III). The maximum effect (ILS, >507% with 50% of the animals surviving for more than 45 days) was noted with treatment on day 0. Treatment on day -1 was still effective (ILS, 207% with two long-term survivors out of six). Treatment on days 1 and 2 showed also remarkable activity with the respective ILS values of 321 and 179%.

Table IV shows antitumor effects against ip-implanted L1210/*ara*-C(I) and L1210/*ara*-C(II) lymphoid leukemia in DBA/2J mice. L1210/*ara*-C(I) was 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 due to deoxycytidine kinase deficiency.[25] 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.[25] Previously, the multiple courses of therapy with *ara*-C (60 mg/kg per day × 15) produced ILS value of 147% among DBA/2J mice bearing ip implanted with 1×10^5 L1210/*ara*-C(I) cells.[25] In contrast, conjugates **8a** and **8b** administered ip as a single dose, in three doses over a 9-day period, or in five doses over a 5-day period were found to be curative in a high percentage of the animals bearing L1210/*ara*-C(I). However, the conjugates were found to be ineffective against ip-implanted L1210/*ara*-C(II) in DBA/2J mice. These results indicated that, like *ara*-C and *ara*-CDP-L-dipalmitin, the *ara*-C conjugates seemed to require deoxycytidine kinase to be effective. Contribution of the thioether lipid moiety to the antitumor activity against L1210 lymphoid leukemia in mice also seemed to be negligible, since the cytidine conjugates **9b** and **9c** did not show any antitumor activity.

Discussion

Two synthetic methods of the thioether lipid intermediates reported in this paper are very significant since they are suitable for the scale-up synthesis of the lipid intermediates. The first one is elimination of column chromatography for purification of the 1-*S*-alkyl-2-*O*-acyl-1-thioglycerols **3a** and **3b** and phosphatidic acids **5a–e**. Compounds **3a** and **3b** were separated conveniently from their isomer 4a and 4b by crystallization in 95% EtOH. The latter were crystallized at room temperature, while the former were crystallized at 0–3 °C. Phosphatidic acids **5a–e** were crystallized in Et_2O at 10–15 °C in pure form. The other significance is use of TBDMS as a protective group for the *sn*-3-OH group in spite of limitations reported previously.[27] Dropwise addition of tetrabutylammonium fluoride at 5–10 °C during deblocking of the TBDMS group minimized the acyl migration.

The conjugates possess two regions, hydrophobic and hydrophilic, in their chemical structures, and thus, they can form micelles in water upon sonication of their suspension. In fact, conjugate **8b** in sonicated solution exists as micellar disks (size 0.01–0.04 μm),[28] which are similar to those formed by *ara*-CDP-L-dipalmitin[29] and CDP-diacylglycerol.[30] The conjugates in micelles were chemically stable during storage at 3–4 °C, and their structure remained intact for a period of more than 6 months.[28] Thus, the conjugates can be formulated safely in micellar solution and the drug formulation is stable in a refrigerator (3–4

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°C). Among the conjugates reported here, conjugate **8b** revealed the strongest antitumor activity. In fact, this agent also displayed a significant therapeutic activity in mice with various solid tumors such as colon 26 carcinoma, M5076 sarcoma,[13] C-1300 neuroblastoma,[14] and 3-Lewis lung carcinoma.[15,16] This agent also inhibited the metastases of M5076 sarcoma to livers[13] and 3-Lewis lung carcinoma to lungs of mice.[16]

The mode of action of the *ara*-C conjugates remains largely unknown. However, their therapeutic effects on L1210 leukemic mice seemed to be mainly due to the *ara*-C moiety, since (1) cytidine conjugates **9b** and **9c** were ineffective (Table III), (2) the *ara*-C conjugates were totally ineffective against deoxycytidine kinase deficient L1210/*ara*-C(II) (Table IV), and (3) administration of **8b** led to a greater intracellular retention of *ara*-CTP than that resulting from *ara*-C.[13] Thus, they may be lipophilic prodrugs of *ara*-C which act as sustained release form of *ara*-C because of the effectiveness by a single dose treatment of **8b** on day -1, 0, 1, and 2 (Table III).

Some other possible pharmacologically favorable properties of the conjugates observed with *ara*-CDP-L-dipalmitin and its analogues include resistance to hydrolysis by cytidine deaminase,[7] rapid interaction with serum lipoproteins,[31] rapid uptake by cells,[32] and effects on lipid biosynthesis.[33,34] Even though effects of the thioether lipid moiety on cytotoxicity against L1210 lymphoid leukemia are not apparent, 1-*S*-alkyl (thioether) analogue **8b** has shown considerably higher efficacy than the 1-*O*-acyl analogue *ara*-CDP–L-dipalmitin against myelomonocytic WEHI-3B leukemia in mice.[35] Thus, the *ara*-C conjugates of 1-*S*-alkyl- (thioether) phospholipids may enhance therapeutic activity by contributing an additional cytotoxic group, 1-*S*-alkyllysophospholipid, after the biotransformation of the lipid moiety according to the biochemical pathways of 1-*O*-alkyl- (ether) phospholipids reported earlier.[36,37] Superior antitumor activity of **8b** compared to that of *ara*-CDP–L-dipalmitin against other animal solid tumor models such as 3-Lewis lung carcinoma[15] may be attributed in part to release of two cytotoxic principles, *ara*-C and 1-*S*-alkyllysophospholipid moieties, from the conjugate.

From our present and previous studies,[10,12] we can only draw the conclusion that the conjugate should be structurally an analogue of the naturally occurring CDP-diacylglycerols which are the immediate biosynthetic precursors to phosphatidylinositol[38,39] and thioether lipid moiety with a long fatty acyl group at the *sn*-2-position seems to improve activity. However, further studies are necessary to elucidate the mode of action of these agents. The enhanced therapeutic activity of conjugates **8a** and **8b** and their broad spectra in both mouse leukemia and solid tumor models warrant further clinical investigation.

Experimental Section

Synthesis. Melting points were taken on a Mel-Temp capillary melting point apparatus and are uncorrected. Nuclear magnetic resonance (^{1}H 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. 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) using 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 detected with a modified Dittmer-Lester spray.[40] The organic compounds were also detected by charring after spraying with the above reagent. Elemental analyses were performed by Galbraith Laboratories, Knoxville, TN and Robertson Laboratory, Madison, NJ. When analyses are reported only by the element symbols, results are within ±0.4% of the theoretical values, including the given numbers of H_2O of hydrations unless noted otherwise. The presence of H_2O in compounds **8b,d,e** and **9b** was confirmed by ^{1}H NMR spectroscopy.

ara-CMP,[41] *ara*-CMP morpholidate,[23] and CMP morpholidate[24] were prepared by a literature procedure. CMP was prepared in a manner analogous to that described for *ara*-CMP.[41]

***rac*-1-*S*-Octadecyl-1-thioglycerol (DL-Thiobatyl Alcohol).** To a mixture of 3-mercapto-1,2-propanediol (DL-1-thioglycerol) (216.3 g 2 mol) in 1000 mL of CH_3OH and stearyl bromide (333.8 g, 1 mol) in 2000 mL of hexanes was added dropwise 2600 mL of 1 N KOH in CH_3OH at room temperature for a period of 1 h and then the mixture was stirred at room temperature for 2 days. The white product was filtered and washed with CH_3OH, 50% aqueous CH_3OH, and CH_3OH (1000 mL of each). The dried product weighed 322 g (89.4%). The filtrate was evaporated to a small volume until the white solid was formed and the additional product was collected on a filter (31.4 g): total yield 98%; mp 76–77 °C (lit.[17] mp 74–75 °C); ^{1}H NMR ($CDCl_3$) δ 0.87 (3, t, J = 6 Hz, CH_3), 1.23–1.50 (32, m, $(CH_2)_{16}$), 2.43–2.67 (4, m, CH_2SCH_2), 3.43–3.80 (3, m, 2-CH, 3-CH_2). *rac*-1-*S*-Hexadecyl-1-thioglycerol (DL-thiochimyl alcohol) was prepared in an analogous manner with cetyl bromide: yield 98%, mp 70–71 °C (lit.[17,18] mp 76–77 °C, 68–70 °C).

***rac*-1-*S*-Octadecyl-3-*O*-trityl-1-thioglycerol (1b, X = C-$(C_6H_5)_3$).** This compound was prepared by a procedure similar to that reported previously.[18] A mixture of 36.1 g (0.1 mol) of *rac*-1-*S*-octadecyl-1-thioglycerol and 30.7 g (0.11 mol) of trityl chloride in 250 mL of anhydrous pyridine was refluxed for 24 h. The solvent was evaporated and the residual pyridine was removed by coevaporation with toluene. The residue was then dissolved in 500 mL of Et_2O, and the ether layer was washed with ice-cold H_2O, 0.5 N H_2SO_4, saturated $NaHCO_3$, and H_2O and dried over Na_2SO_4. The solvent was evaporated, and the residue was dissolved in 300 mL of Me_2CO. After cooling at -10 °C overnight, the solids were filtered and washed with cold Me_2CO. The crude product, essentially homogeneous by TLC, weighed 46.8 g (yield 77.6%) and was used for the next step without further purification: mp 60–62 °C (lit.[18] 64–66 °C). Compound **1a** (X = $C(C_6H_5)_3$) was prepared by using an analogous procedure: yield 73.8%; mp 58–59 °C (lit.[18] 60.5–61.5 °C).

***rac*-1-*S*-Octadecyl-3-*O*-(*tert*-butyldimethylsilyl)-1-thioglycerol (1b, X = TBDMS).** A mixture of *rac*-1-*S*-octadecyl-1-thioglycerol (108 g, 0.3 mol), *tert*-butyldimethylsilyl chloride (49.7 g, 0.33 mol), imidazole (44.9 g, 0.66 mol), and DMF (500 mL)

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was stirred at room temperature for 2 days. 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 140 g (98.2%) and was used for the next step without further purification. Compound **1a** (X = TBDMS) was prepared by using an analogous procedure: yield 100%.

***rac*-1-*S*-Hexadecyl-2-*O*-palmitoyl-3-*O*-trityl-1-thioglycerol (2a, X = $C(C_6H_5)_3$).** To a mixture of 40.2 g (0.07 mol) of **1a** (X = $C(C_6H_5)_3$), 6.7 g (0.084 mol) of anhydrous pyridine, and 150 mL of benzene was added dropwise 21.2 g (0.077 mol) of palmitoyl chloride at room temperature and the mixture was heated at 70–80 °C overnight. The mixture was then mixed with Et_2O and H_2O (500 mL each). The organic layer was separated and washed with 0.5 N H_2SO_4, saturated $NaHCO_3$, and H_2O, dried over Na_2SO_4, and evaporated to dryness. The oily residue was crystallized from a large volume (8 L) of 95% EtOH: yield 46.9 g (82.4%); mp 44–45 °C; ^1H NMR ($CDCl_3$) δ 0.87 (6, t, J = 6 Hz, 2 CH_3), 1.23–177 (54, m, $(CH_2)_{14}$, $(CH_2)_{13}$), 2.23–2.78 (6, m, CH_2SCH_2, CH_2CH_2CO), 3.28 (2, d, J = 4.5 Hz, 3-CH_2), 5.10 (1, m, 2-CH), 6.97–7.47 (15, m, $(C_6H_5)_3$). Compound **2b** (X = $C(C_6H_5)_3$) was prepared in an analogous manner: yield 79%; mp 50–52 °C. Compounds **2c** and **2d** (X = $C(C_6H_5)_3$) were prepared by a literature procedure.[18]

***rac*-1-*S*-Octadecyl-2-*O*-palmitoyl-3-*O*-(*tert*-butyldimethylsilyl)-1-thioglycerol (2b, X = TBDMS).** To a mixture of **1b** (X = TBDMS) (118 g, 0.248 mol), anhydrous pyridine (23.5 g, 0.298 mol), and toluene (500 mL) was added dropwise palmitoyl chloride (75.1 g, 0.273 mol) at room temperature and the mixture was stirred at room temperature for 1 day. The suspension was then partitioned between Et_2O and H_2O (500 mL of 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 (2 L) at 10–15 °C. The solid was filtered and washed with 95% EtOH: yield 173 g (97.8%); ^1H NMR ($CDCl_3$) δ 0.07 (6, s, $(CH_3)_2Si$), 0.88 (15, m, 2 CH_3, $(CH_3)_3C$), 1.23–1.67 (58, m, $(CH_2)_{16}$, $(CH_2)_{13}$), 2.23–2.77 (6, m, CH_2SCH_2, CH_2CH_2CO), 3.75 (2, d, J = 4.5 Hz, 3-CH_2), 4.93 (1, quintet, J = 4.5 Hz, 2-CH_2). The soft solid was essentially homogeneous by TLC and used for the next step without further purification. Compound **2a** (X = TBDMS) was prepared by using an analogous procedure: yield 93.2%.

***rac*-1-*S*-Octadecyl-2-*O*-hexadecyl-3-*O*(*tert*-butyldimethylsilyl)-1-thioglycerol (2e, X = TBDMS).** To a suspension of 8 g (0.2 mol) of 60% NaH in dry THF (200 mL) was added dropwise 47.5 g (0.1 mol) of **1b** (X = TBDMS) in dry THF (200 mL) at room temperature. The mixture was stirred at room temperature for 30 min and cetyl bromide (36.7 g, 0.12 mol) in dry THF (50 mL) was added. The mixture was refluxed overnight and cooled to room temperature. Water (200 mL) was added dropwise to decompose excess NaH, the organic layer was evaporated to dryness, and the oily residue was boiled with 1500 mL of 95% EtOH. After standing at room temperature overnight, the oil was separated and dried in vacuo: yield 52 g (74%). The crude oil product, essentially homogeneous by TLC, was used for the next step without further purification. Compounds **2c** and **2d** (X = TBDMS) were prepared in 95% yield by a reaction of **1b** (X = TBDMS) and CH_3I or C_2H_5I at room temperature in an analogous manner.

***rac*-1-*S*-Octadecyl-2-*O*-palmitoyl-1-thioglycerol (3b). Method A.** To a mixture of **2b** (X = TBDMS) (173 g, 0.24 mol) in HOAc (35 mL) was added dropwise 1 M tetrabutylammonium fluoride in THF (340 mL) for period of 1 h at 5–10 °C and the mixture was stirred at room temperature for 6 h. The mixture was cooled at 0–5 °C overnight and the solid was filtered and washed with 95% EtOH. The solid was mainly product **3b** mixed with the isomer *rac*-1-*S*-octadecyl-3-*O*-palmitoyl-1-thioglycerol (**4b**). The filtrate was evaporated to dryness and the residue was treated with ice-cold 95% EtOH. The solid was a mixture of **3b** and **4b**. The combined solids were dissolved in boiling 95% EtOH (2500 mL) and the solution was cooled to room temperature overnight, which resulted in a white precipitate (mainly **4b**). The solid was removed by filtration (41.7 g, 29%) and the filtrate, which contained mostly **3b**, was cooled at 0–5 °C overnight. The white solid (**3b**) was filtered and washed with cold 95% EtOH. Repeated recrystallization of the crude products in this manner gave 90 g (62.6%) of **3b**: mp 43–44 °C; ^1H NMR ($CDCl_3$) δ 0.85 (b t, J = 6 Hz, 2 CH_3), 1.23–1.67 (58, m, $(CH_2)_{16}$, $(CH_2)_{13}$), 2.38 (2, t, J = 7 Hz, CH_2CH_2CO), 2.60 (2, t, J = 7 Hz, $CHSCH_2CH2$), 2.75 (2, d, J = 7 Hz, 1-CH_2), 3.80 (2, d, J = 4.5 Hz, 3-CH_2), 4.98 (1, quintet, J = 4.5 Hz, 2-CH).

***rac*-1-*S*-Hexadecyl-2-*O*-palmitoyl-1-thioglycerol (3a). Method B.** To a cooled solution of **2a** (X = $C(C_6H_5)_3$) (24.4 g, 0.03 mol) in CH_2Cl_2 (300 mL) was added an equimolar amount of BF_3 (4 g of BF_3–2 MeOH) in one portion at 0 °C. The yellow solution was stirred at 0 °C for 1 h and ice-cold water (200 mL) was added. The organic layer was separated and washed with two additional 300-mL portions of water. The organic layer was dried over Na_2SO_4 and evaporated to dryness. The residue was crystallized from 95% EtOH–hexanes (5:1) at room temperature, which gave isomer **4a** (13.8 g, 80.6%). The filtrate, which contained mainly **3a**, was cooled at 0–5 °C overnight and the solid was collected on a filter, washed with cold 95% EtOH, and dried in vacuo: yield 2.5 g (14.6%); ^1H NMR δ 0.92 (6, t, J = 6 Hz, 2 CH_3), 1.26–1.73 (54, m, $(CH_2)_{14}$, $(CH_2)_{13}$), 2.37 (2, t, J = 7 Hz, CH_2CH_2CO), 2.58 (2, t, J = 7 Hz, $CH_2SCH_2CH_2$), 2.73 (2, d, J = Hz, 1-CH_2), 3.78 (2, d, J = 4.5 Hz, 3-CH_2), 4.97 (1, quintet, J = 4.5 Hz, 2-CH).

***rac*-1-*S*-Octadecyl-2-*O*-palmitoyl-1-thioglycerol 3-Phosphate (5b). Method C.** To an ice-cold mixture of $POCl_3$ (44.5 g, 0.29 mol) and hexanes (100 mL) was added dropise triethylamine (29.35 g, 0.29 mol) in hexanes (100 mL). To this mixture was added dropwise a solution of dried **3b** (111 g, 0.19 mol) in toluene (600 mL) at 0–5 °C over a period of 1.5 h, and then the mixture was stirred at room temperature overnight. Water (100 mL) was added to the mixture and the suspension was stirred at room temperature for 1 h. The mixture was then partitioned between Et_2O (1000 mL) and H_2O (500 mL). The organic layer was dried over Na_2SO_4 and evaporated to dryness. The residue was crystallized from hexanes at 0–5 °C and then recrystallized from Et_2O at room temperature: yield 57.7 g (44.7%); mp 65–67 °C; ^1H NMR δ 0.87 (6, t, J = 6 Hz, 2 CH_3), 1.25–1.65 (58, m, $(CH_2)_{16}$, $(CH_2)_{13}$), 2.37 (2, t, J = 7 Hz, CH_2CH_2CO), 2.60 (2, t, J = 7 Hz, $CH_2SCH_2CH_2$), 2.73 (2, d, J = 7 Hz, 1-CH_2), 4.17 (2, m, 3-CH_2), 5.12 (1, m, 2-CH). Phosphatidic acids **5a,c–e** in Table I were prepared in an analogous manner. They were homogeneous by TLC and used for the condensation with *ara*-CMP morpholidate (**6**) and CMP morpholidate (**7**) without elemental analyses.

***ara*-CDP-*rac*-1-*S*-octadecyl-2-*O*-palmitoyl-1-thioglycerol (8b).** Compound **5b** (26.48 g, 0.039 mol) was dried azeotropically with pyridine three times and mixed with *ara*-CMP morpholidate (**6**) (21.43 g, 0.031 mol) followed by coevaporating with pyridine two times. The dried mixture was then mixed with anhydrous pyridine (1500 mL) and stirred at room temperature for 10 days. The solvent was evaporated and the residue was coevaporated with toluene to remove the residual pyridine. The residue was dissolved in 2000 mL of $CHCl_3$–MeOH–H_2O (2:3:1) and then mixed with 200 mL of 0.2 N HCl. The organic layer was separated and the aqueous layer was extracted with $CHCl_3$ (2 × 100 mL). The combined organic layers were evaporated to dryness and the residue was dissolved in 3000 mL of $CHCl_3$–MeOH–H_2O (2:3:1). The solution was then applied to a DE-52 (AcO^-) column (10 × 30 cm) prepacked with the same solvent. The column was eluted with $CHCl_3$–MeOH–H_2O (2:3:1) (3500 mL) and then with 0.04 M NH_4OAc in the same solvent. The 0.04 M NH_4OAc fractions between 11 000–16 500 mL 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 **8b**) was dissolved in $CHCl_3$–MeOH–H_2O (2:3:1) and the solution was passed through a CG-50 (Na^+) column (5 × 20 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, which was **5b**. The filtrate was evaporated to a small volume and the product (Na salt) was filtered, washed with acetone, and dried in vacuo: yield 24.13 g (37.85%); mp 206–207 °C; ^1H NMR ($CDCl_3$–CD_3OD–D_2O, 2:3:1) δ 0.92 (6, t, J = 6 Hz, 2 CH_3), 1.30–1.77 (58, m, $(CH_2)_{16}$, $(CH_2)_{13}$), 2.32 (2, t, J = 7 Hz, CH_2CH_2CO), 2.52 (2, t, J = 7 Hz, $CH_2SCH_2CH_2$), 2.72 (2, t, J = 7 Hz, 1-CH_2), 3.93–4.58 (7, m, 3-CH_2, H-2′, H-3′, H-4′, H-5′), 5.08 (1, m, 2-CH), 5.94 (1, d, J = 7.5 Hz, cytosine H-5), 6.11 (1, d, J = 5.25 Hz, H-1′), 7.84 (1, d, J = 7.5

Hz, cytosine *H*-6). Conjugates **8a,c–e** and **9b,c** in Table II 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.

Antitumor Activity in Vivo. DBA/2J male mice in groups of six (wt 20–30 g) were inoculated ip with 1×10^6 L1210/0 or 1×10^5 L1210/*ara*-C(I) or L1210/*ara*-C(II) lymphoid leukemia cells,[26] and a sonicated solution of the conjugates was given ip as reported earlier.[12] Each drug was tested over a wide range of doses. The results from the representative dose levels are shown in Tables III and IV.

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Registry No. **1a** (X = H), 25666-00-6; **1a** (X = TBDMS), 125592-12-3; **1a** (X = Tr), 103321-06-8; **1a** (X = R_1 = H), 53023-42-0; **1b** (X = H), 25666-01-7; **1b** (X = TBDMS), 95244-99-8; **1b** (X = Tr), 91274-06-5; **2a** (X = TBDMS), 125592-15-6; **2a** (X = Tr), 103612-81-3; **2b** (X = TBDMS), 125592-14-5; **2b** (X = Tr), 125592-13-4; **2c** (X = TBDMS), 125592-17-8; **2d** (X = TBDMS), 125592-18-9; **2e** (X = TBDMS), 125592-16-7; **3a**, 103612-83-5; **3b**, 125592-19-0; **3c**, 103304-70-7; **3d**, 103304-71-8; **3e**, 125592-22-5; **4a**, 103612-82-4; **4b**, 125592-20-3; **5a**, 103612-84-6; **5b**, 125592-21-4; **5c**, 125592-23-6; **5d**, 125592-24-7; **5e**, 125592-25-8; **6**, 125592-26-9; **7**, 87713-33-5; **8a**·2Na, 125592-29-2; **8b**·2Na, 125592-28-1; **8b**·$2NH_3$, 125592-27-0; **8c**·2Na, 125592-30-5; **8d**·2Na, 125592-31-6; **8e**·2Na, 125592-32-7; **9b**·2Na, 125592-33-8; **9c**·2Na, 125592-34-9; stearyl bromide, 112-89-0; cetyl bromide, 112-82-3; palmitoyl chloride, 112-67-4.

Cytotoxicity and Antitumor Activity of Some Tetrahedral Bis(diphosphino)gold(I) Chelates[1]

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We report the cytotoxicity toward B16 cells and antitumor activity in three transplantable tumor models of a series of ionic, tetrahedral, bischelated gold diphosphine complexes of the type $[Au^I(R_2PYPR_2')_2]X$, where Y = $(CH_2)_2$, $(CH_2)_3$, or *cis*-CH=CH. The anion (X = Cl, Br, I, CH_3SO_3, NO_3, PF_6) had little effect upon activity. The R = R′ = phenyl complexes 1, 7, and 8 [Y = $(CH_2)_2$, $(CH_2)_3$, *cis*-CH=CH, X = Cl] were the most active against P388 leukemia, with an increase in lifespan ranging from 83 to 92% and were also active against M5076 sarcoma and B16 melanoma. Complexes with pyridyl or fluorophenyl substituents had reduced activities. For the latter, ^{19}F and ^{31}P NMR were used to verify the formation of bischelated gold(I) complexes in solution. The reduced activity of the complex with R = Et and R′ = Ph and inactivity with R = R′ = Et are discussed in terms of their increased reactivity as reducing agents. ^{31}P NMR studies show that $[Au^I(Et_2P(CH_2)_2PPh_2)_2]Cl$ readily reacts with serum, albumin, and Cu^{2+} ions to give oxidized ligand.

The linear two-coordinate triethylphosphine gold(I) complex auranofin (1-thio-β-D-glucopyranose-2,3,4,6-tetraacetato-*S*)(triethylphosphine)gold(I), "Ridaura", Smith Kline and French Laboratories) is an orally active antiarthritic agent[2] with in vitro antiproliferative effects against B16 melanoma cells and P388 leukemia cells as well as cultured human cancer cells.[3,4] It is active against intraperitoneally (ip) implanted P388 leukemia in mice,[5] but only when administered ip, and it is inactive in other tumor models.[6] This restricted range of activity may be related to facile ligand-exchange reactions which auranofin can undergo. In plasma and in cells the tetraacetyl-β-D-thioglucose ligand is readily displaced by other thiolate ligands, and the phosphine ligand can be released and undergo oxidation to the oxide.[7–9]

We have recently prepared stable four coordinate gold(I) diphosphine complexes.[10,11] These ionic, chelated, tetrahedral complexes exhibit a spectrum of chemical activity different from that of auranofin. For example, they do not readily react with thiols.[12] Moreover extensive testing of bis[1,2-bis(diphenylphosphino)ethane]gold(I) chloride, ($[Au(dppe)_2]Cl$, 1) showed that it was active in several tumor models.[12] Further interest in the tetrahedral complexes has arisen from the observation that the bridged digold complexes $[\mu\text{-}Ph_2P(CH_2)_nPPh_2]Au^I_2X_2$ (A, X = Cl or β-D thioglucose) readily undergo rearrangement reactions in the presence of thiols or blood plasma to give

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